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AUSTRALASIAN ANNALS OF MEDICINE

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HOMINUM SERVIRE SALUTI

I will use treatment to help the sick according to my ability and judgment, but I will never use it to hurt or wrong them.

HIPPOCRATIC OATH.

The principal grievance that I have against the doctors is that they neglect the real problem which is to seize the unity *of the individual who claims their care : their methods of investigation are far too elementary ; a doctor who does not read you to the bottom is ignorant of essentials. To me, the ideal doctor would be a man with profound knowledge of life and of the soul, intuitively divining any suffering or disorder of whatever kind, and restoring peace by his mere presence.*

H. F. AMIEL (1873).

THE Hippocratic physicians lived in an age no less challenging than our own. They established the proper practice of careful clinical observation, and they initiated the emancipation of therapeutics from the religious, magical and supernatural influences previously invoked. The section of the Hippocratic Oath concerning treatment was included not as a censure of past practice, but as a statement of principle for future guidance. Their suggested code of conduct arose from a sincere desire to do good, rather than from any fear of penalties for error such as had been imposed on earlier Babylonian physicians in the reign of Hammurabi.

In the minds and practice of conscientious physicians, this principle of *non nocere* has well withstood the passage of time. The infrequent defections have served to emphasize the traditional benevolence and high standards of the medical profession as a whole. During the Dark and Middle Ages, many patients were the victims of errors of judgement in clinical trials of new remedies ; but such mistakes arose from ignorance or misguided enthusiasm rather than intent. The employment of chemical substances rather than herbal medicines started with the ancients, but did not reach significant proportions until the Middle Ages. Opinion was so sharply divided about the propriety of the use of such drugs that, in 1615, the Royal College of Physicians of Paris passed a decree in which chemical medicines were condemned and interdicted from all pharmacopœias, and all judges were implored " to inflict severe chastisement on all who prescribed, administered and exhibited these poisonous substances ". Then, as now, public demand for the newest therapy influenced clinical practice. As one cynic said at the time in relation to Sylvius, who used antimony freely, it seemed " better far that the patient should die in the fashion rather than live according to the wisdom of the ancients ".

Up till the end of the nineteenth century, effective remedies available were so few that physicians often sought to impress by personality, by pontification, by elegant polypharmacy or by seeking to disguise their therapeutic impotence by some action usually unphysiological and invariably unavailing. Looking back, it appears that a surfeit of

the art of medicine attempted to hide the defects of the science, and it seems almost inconceivable that Trousseau should have had to exhort his students : " For mercy's sake, gentlemen, let us have a little less science and a little more art." Today, an abundance of the science certainly tends to overshadow and may even imperil the proper practice of the art. The modern physician, endlessly exploring the physico-chemical basis of life itself, accepts with increasing faith and only passing question a great and rapidly increasing number of chemical substances which have been shown by experiment and proved by experience to be capable of therapeutic effects. He acknowledges his increased power to do good, but is equally aware of his mounting capacity to do harm. Even the strict clinical trials which precede the release of new drugs do not protect the patient from idiosyncrasy, sensitivity or unexpected side effects. In many cases the physician elects to take a known and calculated risk, on the assumption that the prospect of benefit to the patient is judged to be immeasurably greater than the chance of injury.

When accepted drugs in approved dosage injure the patient, conscience alone can guide or chide the physician. It is said that conscience in all things is educable ; but in medicine conscience cannot be salved solely by claiming conformity with accepted standards. Professional conscience has always maintained standards in advance of the *mores* of society : the individual physician's conscience, in dealings with human beings, is governed further by a sense of obligation and of " oughtness ".

The conscientious physician, seeking " *hominum servire saluti* ", is aware that responsibility for drug therapy is but a small part of his total duty to the patient. He interprets " *salus* " in its fullest sense of wholeness, well-being and physical health, and may seek guidance in some simple, practical philosophy, such as " *guérir quelquefois, soulager souvent, conforter toujours* ". The patient usually regards the visible and tangible results of his interview as the only " treatment " ; he is less aware that a physician " treats " not only by what he says and does, but by what he is. The physician knows, consciously or subconsciously, that he treats the patient, for good or ill, by his manner, by his presence or his absence, by his utterance or his silence, by his wisdom or his ignorance, by the care of his interrogation and the completeness of his examination. In many cases he treats most by his capacity to create an atmosphere of timeless, single-minded attention, an attitude of positive listening which forms, as it were, a vacuum into which, almost involuntarily, the patient releases the symptoms of his body and the torments of his mind. These subtle influences of the physician's eye and heart and hand cannot be scientifically assessed, but they reach their full expression in the complete *rapport* of two personalities—one seeking help and the other desiring to give it. The physician aspires to the management of people rather than the treatment of disease alone ; his contact with patients has a content of feeling as well as of trained observation and applied science. The fullest revelation may come from a sensitive silence rather than a searching question. A slight quiver of a lip, a glint of restrained tears, a sudden reticence, a reaction to examination may tell the physician more than the patient can express in words or than medical science can reveal in tests. The whispering voice of intuition, arising in subconsciously stored experience, may guide when all else fails. In " treatment " patience, understanding and compassion prepare the ground, perception enriches it, knowledge sows the seed, the perfection of the art of medicine ripens the full harvest.

The art of medicine is difficult to define ; it cannot be measured, it can only be valued. It cannot be taught ; it can be acquired only by experience and by a development of those aptitudes referred to as a " natural disposition " in Hippocratic writings. It was classified by Wilfred Trotter as one of the practical arts ; but it differs from other practical arts in that the material upon which the artist works is human and, as such, is entitled to subjective

opinions about the techniques of the artistry and the merits of the product. It might be expected or hoped that the advance of the science of medicine would reduce the need for the practice of the art; in fact, the reverse is the case. The art, which is inexact but inherently conservative, gives stability to medicine in a rapidly changing scientific environment. Today the art is required to humanize the impact of medical science on the patient; tomorrow it may be called upon to protect the patient from depersonalized automation.

In undertaking the management of the patient as well as specific drug therapy, the physician shoulders heavy responsibilities. He receives the patient's trust, calms his fears, resolves his troubles, stands as a symbol of hope and may have to act as a guardian of faltering faith or as an architect of a new design for living. Guided by a more positive philosophy than *non nocere*, he does not retreat to therapeutic nihilism in the knowledge that the drugs he prescribes may do harm, but he remains constantly aware that he may inflict the greatest injury by neglect of the art of medicine. The frequency of failure of the art in the past is revealed in the coining of the term "iatrogenic illness" by Sir Arthur Hurst before the days of modern drugs. Modern scientific medicine has to be interpreted to the patient; this involves careful consideration, not only of what the physician says and does, but also of what the patient and those about him understand and feel about such words and actions. Medical justice must be done, and must seem to the patient to be done. Medical science has added years to life; but it remains for the art of medicine to add life to the years. Positive rehabilitation is a routine of permissions and encouragements. The inadequate explanations and the negations of rigid or thoughtless practice have always been productive of cumulative fears and unnecessary functional illness. Guidance of the patient towards the acceptance of disability and the understanding of his treatment is as important as the treatment itself. There may be times when the best treatment is little or none in terms of the science, but much in terms of the art. It would be a backward step if a new generation of scientifically trained and equipped physicians were ever tempted to prescribe more and treat less. In terms of human misery, neglect of the art of medicine may contribute more offence than misadventure in the application of the science.

The motto of the College serves as an inspiration for all physicians who give thought to the content of their professional purpose and to their success in attaining it. The standards towards which the College aims and guides demand equal competence in the science and in the art of medicine. The physician will serve most who has a knowledge of the science, a devotion to the art and the wisdom to combine both in the best interests of each individual patient. Amidst the perplexities of the physician's growing responsibilities, "reverence for life" will always be cherished as an ideal. Albert Schweitzer, who so well shaped his professional life round this philosophy, has, with the simplicity of true greatness, lighted a beacon on his wharf at Lambaréné which may well, in its fullest literal and metaphorical sense, illumine the practice of every physician. In the middle of a hostile jungle filled with danger, disease, fear and suffering, there shine these words:

> Here, at whatever hour you come, you will find light and help and human kindness.

WILTON HENLEY.

THE CARDIOMYOPATHIES : A REPORT OF FIFTY CASES[1,2]

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SUMMARY

" The cardiomyopathies " is the term applied to an ill-defined group of cardiac disorders which have in common a non-coronary, non-rheumatic, non-hypertensive myocardial fault. They are not rare and should be considered in any unusual cardiac state. They may present by sudden death, with acute or chronic cardiac failure, with arrhythmias or with purely electrocardiographic changes. The clinical picture is classically one of pulmonary œdema or gross right-heart failure, usually with a low output state. The X-ray film does not show any distinguishing features, and the electrocardiogram most commonly shows *T*-wave changes.

In the present series there are two congenital groups, 10 cases in all—one associated with the hereditary ataxias and muscular dystrophies, and a second in which there is a predominant endocardial or myocardial abnormality or a combination of both—for example, fibroelastosis, idiopathic hypertrophy and endomyocardial fibrosis. Endomyocardial fibrosis may also be an acquired disorder. Myocarditis made up the largest group in the acquired series (19 cases). It may be associated with general or upper respiratory tract infections, with parasitic infestations or with pregnancy, or it may follow operation. There is a high incidence of sudden death. Collagen diseases (nine cases) may present in this way with cardiac failure the outstanding feature. This may respond to steroid therapy. Metabolic disorders (12 cases), such as primary amyloidosis, hæmochromatosis and alcoholic cardiomyopathy, have also been included. There is much need for progress in this field.

IN recent years, as one has asked critically why each patient has cardiac disease or cardiac failure, it has become obvious that there is a group of disorders with a primary myocardial fault which is non-rheumatic, non-coronary and non-hypertensive. The importance of this primary myocardial fault was emphasized by Christian (1950), and these uncommon myocardial diseases have been called myocarditis and myocardosis by Blankenhorn and Gall (1956), according to whether there is infection present or not. Wood (1956) terms this group non-rheumatic myocarditis and cardiopathies, and Brigden (1957) suggested the term cardiomyopathies. This group is commoner than is realized, and it is probable that clinicians tend to under-diagnose these cases, while enthusiastic pathologists such as Saphir (1951) have perhaps over-stressed their frequency. This report is concerned with 50 cases, of which the diagnosis has been confirmed at autopsy in 34 and at operation in one. Cases of collagen disease have been included only when the cardiac manifestations were marked or predominant. Their inclusion serves to emphasize the necessity of considering such a possibility in obscure cardiac failure.

The series is made up as shown in Table I.

GENERAL CLINICAL FEATURES

The commonest mode of presentation was with congestive cardiac failure, which was present in 34 cases. This usually began as exertional breathlessness, with gradual progression to gross cardiac failure. In 15 cases pulmonary œdema occurred at some stage, and in five it was the presenting symptom. This is much higher than in the series reported by Brigden (1957), and may be accounted for by the high percentage of cases of myocarditis in this series. Weakness and tiredness, particularly on effort, were commoner than is usual in cardiac failure and were a marked feature in 11 cases. Precordial pain of cardiac type was present in eight cases; it differed from the pain in coronary artery disease, in that it usually started some time after the onset of breathlessness. Pain in the right hypochondrium occurred in eight cases, and in three this was a constant complaint even when the patient was at rest. These were all patients with alcoholic

[1] Received on February 26, 1960.

[2] Read at the plenary session of The Cardiac Society of Australia and New Zealand at the annual meeting of The Royal Australasian College of Physicians, May 27, 1959.

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cardiomyopathy. Palpitation was a prominent feature in seven patients, of whom three were alcoholics, two had myocarditis and one had disseminated lupus erythematosus. Syncope on exertion occurred in one patient with primary amyloidosis.

Sudden death was the mode of presentation in five cases of myocarditis. In the cases of myocarditis, the symptoms progressed rapidly over a few days or weeks. Those patients who are still alive have chronic cardiac failure or have slowly improved over many months.

TABLE I

Cardiomyopathies (50 Cases)

Type or Ætiology	Number of Cases
Congenital:	
Hereditary ataxias	4
Muscular dystrophy	3
Fibroelastosis (endomyocardial fibrosis)	1
Familial cardiomegaly	1
Idiopathic hypertrophy	1
Myocarditis:	
Non-specific: Acute 4, Subacute 2	6
Acute virus infection	1
Diphtheria	2
Acute post-operative	3
Pregnancy	5
Chronic on acute	1
Parasitic—hydatid disease	1
Collagen diseases:	
Polyarteritis nodosa	4
Disseminated lupus erythematosus	3
Scleroderma	2
Metabolic disorders:	
Primary amyloidosis	2
Hæmochromatosis	1
Alcoholic cardiomyopathy	8
Undetermined	1

The other patients in this series have progressed slowly, with a course made worse by arrhythmias and/or embolic phenomena. The response to therapy has been poor, particularly in the alcoholic group.

Electrocardiographic changes were the most significant cardiac feature in three cases of hereditary ataxia, in one case of post-diphtheritic myocarditis, and in one case of acute virus infection.

Clinical Signs

Signs may not always be obvious, but when marked, they are classical. The jugular venous pressure was grossly raised in 28 cases, with a marked pulsation due to a large CV wave or a striking Y descent. On inspiration, the level of the venous pressure rose in four cases as in constrictive pericarditis. In the 34 cases in which cardiac failure developed, œdema and hepatomegaly were present. The latter may be very persistent and cause marked pain. Twenty patients had a small pulse pressure and cold, cyanosed extremities. No patient in this series showed any evidence of a high output state. A systolic murmur was audible in one-third of the cases, and a triple rhythm occurred at some stage in half. Pulmonary emboli occurred in five cases, and in three others there were peripheral emboli. At autopsy on seven patients there was extensive antemortem clot in the left ventricle, and in one patient in the right ventricle.

A full clinical examination is important in the assessment of these patients and as an aid to diagnosis. In one case a palpable spleen suggested lupus erythematosus, and in another gross albuminuria led to a diagnosis of primary amyloidosis. Often, however, a full examination did not aid the diagnosis.

Radiological Findings

Radiographic examination of the chest may show generalized cardiac enlargement with no distinguishing features. Pulmonary congestion was variable, but was usually not marked.

Electrocardiographic Findings

The electrocardiogram was always abnormal in the 37 cases in which it was available. The *P* wave was abnormal in seven, and in six it suggested right atrial enlargement. The *P-R* interval was prolonged in one case of progressive muscular dystrophy. A nodal rhythm occurred in one case of hereditary ataxia. The commonest change was widespread flattening or inversion of the *T* wave (Figure I), which occurred in 28 cases. In eight cases there were *S-T* segment changes without *T*-wave alteration. *Q* waves were present in seven cases, usually over the right ventricle or transitional zone, so that the electrocardiogram was often reported as showing an old anterior myocardial infarct (Figure II). Left bundle branch block was present in five cases. Auricular fibrillation occurred in four, auricular flutter in one, and frequent ventricular premature beats in four, with runs of ventricular tachycardia in one. In one case of disseminated lupus erythematosus, classical features of a recent anterior myocardial infarct were present. Right bundle branch block or right ventricular hypertrophy was present in two cases in this series; these patients were a male, aged 51 years, with scapulo-humeral muscular dystrophy, and a male, aged 31 years, with hæmochromatosis. Weisenfeld and Messinger (1952) found tall *R* waves in leads V1 and V2 in 13 of 18 cases of progressive muscular dystrophy.

Investigations

Cardiac catheterization is usually contra-indicated for patients with cardiomyopathy, because of the large heart, the frequent ventricular premature beats and the presence

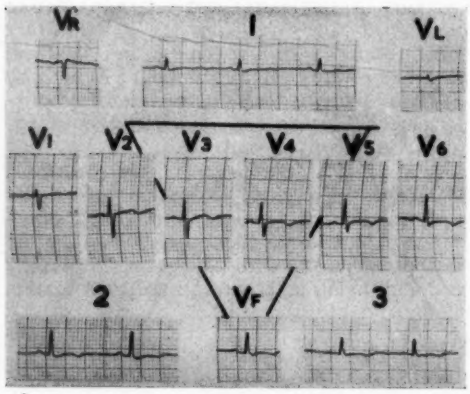


FIGURE I
Electrocardiogram of a patient with myocarditis showing widespread *T*-wave changes

of large *Q* waves. It was performed in two cases of pregnancy myocarditis after the cardiac failure had been controlled, and in one case of post-diphtheritic myocarditis. It revealed

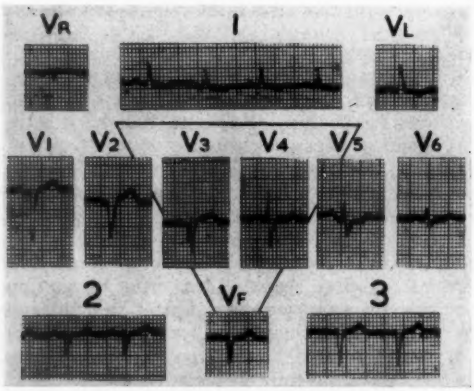


FIGURE II
Electrocardiogram of a patient with primary amyloidosis showing *Q* waves in leads V2 and V3. This had been reported as an old anterior myocardial infarct.

normal hæmodynamics. Hetzel *et alii* (1953) have pointed out that constrictive pericarditis and myocarditis may give similar right ventricular pressure tracings. Other investigations have included a full blood count, estimation of the erythrocyte sedimentation rate, examination for lupus erythematosus cells, estimation of the serum cholesterol level, an electrophoretic curve, a liver function test, a Congo red test, and skin, gum, renal or hepatic biopsy. In this series these tests were only occasionally helpful.

CONGENITAL CARDIOMYOPATHY

Associated with the Hereditary Ataxias

In this series there were three sisters, aged 35, 39 and 43 years, from a family of five (Figure III). The other two siblings were normal males, and there was no relevant family history. The eldest of the affected women had palpitations and breathlessness, but the others were symptomless. Clinically and radiologically the heart was found to be displaced, because of scoliosis, in all three ; but in the eldest there was left ventricular enlargement.



FIGURE III
Three sisters with hereditary ataxia and cardiac involvement

The fourth patient was a male, aged 24 years. He had had exertional breathlessness, precordial pain and palpitations for twelve months. Clinically and radiologically his heart appeared normal except for frequent extrasystoles. An electrocardiogram showed nodal rhythm, auricular premature beats and widespread *T*-wave inversion.

Lorenz *et alii* (1950) have stressed that cardiac involvement is a serious and relatively frequent concomitant of Freidreich's disease, and Evans and Wright (1942) found electrocardiographic changes in 12 of 38 patients. The commonest abnormalities were *T*-wave changes, but complete heart block and left bundle branch block occurred. At autopsy, muscle necrosis, compensatory hypertrophy of muscle fibres, fatty degeneration, interstitial fibrosis and foci of cellular infiltration make up a picture of chronic interstitial myocarditis which leads to heart failure (Russell, 1946).

Associated with Muscular Dystrophies

There were two brothers, aged 15 and 16 years, with progressive muscular dystrophy. They had one normal sister, and there was no relevant family history. The younger died from uncontrollable heart failure, while the elder had marked muscular wasting and cardiac failure. Both had gross cardiac enlargement, and the electrocardiograms showed widespread *T*-wave changes. In one case there was a prolonged *P-R* interval, and, later, auricular fibrillation.

The third patient in this group was a male, aged 51 years, with scapulo-humeral muscular dystrophy and progressive dyspnœa, cardiac pain and gross right-heart failure. The breathlessness preceded the muscular symptoms by three years. An electrocardiogram showed auricular fibrillation, right bundle branch block and later *Q* waves in leads V1 and V2. A deltoid muscle biopsy showed necrotic muscle bundles interspersed with apparently normal fibres.

Rubin and Buchberg (1952) found cardiac involvement in 50% of cases of progressive muscular dystrophy, while Weisenfeld and Messinger (1952) suggested a figure of 80%. Grandell (1956), on electrocardiographic and ballistocardiographic evidence, thinks the frequency has been over-stated. The cardiac involvement may lead to no clinical manifestations, to cardiac failure, to tachycardia or to arrhythmias. The last-mentioned are particularly frequent (Zatuchni *et alii*, 1951). The cardiac disturbances play an important role in the terminal phase of the disease, particularly in younger patients (Weisenfeld and Messinger, 1952). The juvenile or pseudo-hypertrophic type usually ends fatally with cardiac failure at about the age of 20 years. The facio-scapulo-humeral type is compatible with longer life, even extending into the sixth decade, when cardiac failure occurs (Lisan *et alii*, 1959). This is seen in our third case. The majority of patients are males, and systolic murmurs have occasionally been heard in the pulmonary area (Bevan, 1945). The histological picture is one of muscular atrophy, fibrous tissue replacement, hypertrophy of muscle fibres, fatty infiltration and collections of lymphocytes (Weisenfeld and Messinger, 1952). The electrocardiogram may show right-sided preponderance, but may show almost any abnormality, including pathological *Q* waves (Rubin and Buchberg, 1952). Cardiac involvement is not found in any of the other muscular dystrophies except dystrophia myotonica (Sandberg *et alii*, 1952). This involvement occurs in 50% to 60% of reported cases, and is usually manifested by electrocardiographic abnormalities (Kilpatrick and Caughey, 1955). At post-mortem examination the heart may show little or no abnormality, or there may be adipose and collagenous replacement of myocardial fibres. Such patients are particularly prone to respiratory troubles and death under "Pentothal" anæsthesia (Pachomov and Caughey, 1958).

Fibroelastosis

There was one patient with fibroelastosis, a male, aged 53 years, who presented with a low output state, gross right-heart failure, a right pleural effusion, and frequent ventricular premature beats. Death occurred within twelve months, and autopsy showed normal coronary arteries and a dilated, hypertrophied heart. The endocardium of both ventricles, particularly the left, was markedly thickened, and mural thrombus was present in the left ventricle. Examination of sections showed gross endocardial thickening with increased elastic tissue (Figure IV). There were only occasional areas of fibrosis in the myocardium.

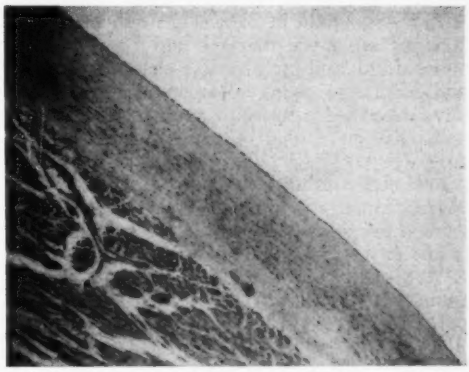


FIGURE IV

Photomicrograph of the left ventricle, showing gross endocardial fibroelastosis. (Hæmatoxylin and eosin stain. ×90)

Thomas *et alii* (1954) have pointed out that instances of this disorder can be divided into three groups on an age basis—infantile, childhood and adult. The infantile variety causes sudden death or rapidly fatal heart failure in infancy (Dennis *et alii*, 1953). At autopsy there is advanced generalized endocardial thickening, with proliferation of both collagenous and elastic tissue and hypertrophy of the myocardium. These changes are most marked in the left ventricle (Prior and Wyatt, 1950). In 52% of cases a deformity of the aortic or mitral valves is present (Dennis *et alii*, 1953).

The childhood group is identical with the infantile group, except that it occurs from two to 16 years of age. There is a lesser degree of endocardial thickening with less striking hypertrophy. A latent period occurs before the onset of symptoms. It is thought that these changes are congenital in origin, and are due either to persistence and overgrowth of the primitive lining of the left bulbus cordis (Dennis

et alii, 1953) or to intrauterine endocardial anoxia from premature closing of the foramen primum or ovale (Johnson, 1952).

The adult variety presents as chronic cardiac failure, with mural thrombosis and embolic phenomena a prominent feature. The fibroelastic endocardial thickening may be generalized or patchy, and associated with patchy myocardial fibrosis (Thomas *et alii*, 1954). The myocardial fibrosis may be marked (Panke and Rottino, 1955). It may occur at any age, even in the elderly (White and Fennel, 1954). In recent years several series of cases of extensive endocardial and subendocardial fibrosis have been described. In some there is a distinct demarcation of the endocardium (Thomas *et alii*, 1954), while in others the subendocardial changes are more marked and fuse with the endocardial and myocardial changes (Bedford and Konstam, 1946). Davies and Ball (1955) have described a series of cases in the Uganda, and O'Brien (1954) a series in the Sudan. Any age was affected, but the condition was commonest in middle-aged and elderly patients. No inflammatory, allergic or nutritional ætiology seemed to explain the lesions. However, Becker *et alii* (1953) hold that endomyocardial fibrosis is a diffuse collagen disease, and Watt and Lynch (1956) have described the lesion in polyarteritis nodosa.

Familial Cardiomegaly

There was one patient with familial cardiomegaly, a boy, aged six years, whose sister had died suddenly at the same age. He dropped dead on the way to school. Autopsy showed cardiac hypertrophy, extensive myocardial fibrosis and compensatory hypertrophy of muscle fibres. There were no other abnormalities.

Evans (1949) has described the syndrome of familial cardiomegaly. These patients often have few symptoms, but ultimately develop palpitation, giddiness, Stokes-Adams attacks, paroxysmal tachycardia, auricular fibrillation and heart failure, and die suddenly. The electrocardiogram usually shows a wide *QRS* complex with *T*-wave inversion. The prognosis is poor, and fibrosis of the myocardium with hypertrophy of the muscle fibres is conspicuous. Gaunt and Lecutier (1956) have reported a similar family, in one member of which large deposits of glycogen were found in the heart.

Idiopathic Hypertrophy

In this series there was a male patient, aged 33 years, who presented and died with acute pulmonary œdema without hypertension. An electrocardiogram showed a left bundle-branch block, and autopsy revealed classical macroscopic and microscopic features of right and left ventricular hypertrophy. The coronary arteries and all other organs were normal.

Wood (1956) states that idiopathic hypertrophy is not an entity in itself; but Levy and von Glahn (1944) found hypertrophy the only microscopic change in four of a series of 10 young adults with cardiac enlargement and heart failure of unknown ætiology. Teare (1958) has described nine cases of sudden death, in which there was asymmetrical hypertrophy or muscular hamartoma.

MYOCARDITIS

Myocarditis made up the largest group in this series, there being 19 cases in all. These have been classified as in Table I. The term has been restricted to those cases in which there was reasonable evidence that the lesion was closely related to infection. Gore and Saphir (1947) have shown that myocarditis may occur in association with most of the known infectious diseases. Often the myocarditis may be insignificant and its frequency may have been over-stated, particularly when this is based on electrocardiographic changes. Fine *et alii* (1950) found abnormal electrocardiograms in 33% of cases of acute infectious disease. Saphir (1942) places the incidence at 4% in any group of infectious diseases.

Cardiac pain was not a prominent feature of the patients with myocarditis. It occurred in two of the women (with pregnancy myocarditis and in one patient with chronic myocarditis. Gillis and Walters (1954) have described myocarditis simulating coronary occlusion.

Acute Myocarditis

The acute myocarditis group consisted of four cases which presented by sudden death or by rapidly progressive heart failure over 24 hours.

A typical example was a Chinese, aged 35 years, who dropped dead whilst carrying a load on to a ship. Examination of sections of his heart showed patchy necrosis of the muscle with focal infiltration with lymphocytes and polymorphs (Figure V).

The high incidence of sudden death in apparently healthy persons has been stressed by Moritz and Zamchek (1946) and by Bowden (1950).

Subacute Myocarditis

The subacute group differed only in the speed of onset and progression of the symptoms. Fatigue was a prominent feature. This group comprised two patients, a male, aged 39 years, and a boy, aged 11 years, and the histories covered five months and one month respectively. In both cases, the histological changes were indistinguishable from those in the acute group, but there was ante-mortem clot in the ventricles

in both cases, and in one a peripheral embolus caused the patient's admission to hospital. De la Chappelle and Graef (1931) have described a cerebral embolus as the mode of presentation in a case of myocarditis.

Fiedler's (1899) name is classically associated with myocarditis causing cardiac failure and extending over weeks or months. He described five cases in young adults. It is probable that this group of isolated or idiopathic myocarditis is becoming smaller and may be due to any infectious disease, particularly viruses. Recently it has been described in association with Cocksackie virus (Javett *et alii*, 1956), with

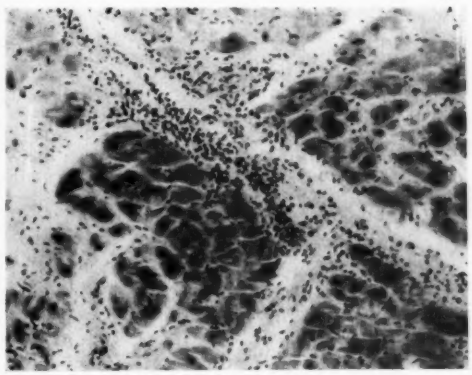


FIGURE V

Photomicrograph of the myocardium showing interstitial infiltration with inflammatory cells, predominantly polymorphonuclear neutrophils. (Hæmatoxylin and eosin stain. ×180)

poliomyelitis (Spain *et alii*, 1950), with influenza A (Finland *et alii*, 1945; Gibson *et alii*, 1959), with infectious mononucleosis (Evans and Graybiel, 1946) and with infectious hepatitis (Dehn *et alii*, 1946). Two of the above-mentioned six patients had a history of upper respiratory tract infection. This has previously been stressed by Lustok *et alii* (1955), who found it in one-third of 45 cases.

Myocarditis Associated with Virus Infection

One patient, a female, aged 18 years, presented with syncope, frequent ventricular premature beats and electrocardiographic *T*-wave changes. Virocytes were present in the peripheral blood, and her sister had recently had infectious hepatitis. She recovered with bed rest and quinidine, and her electrocardiogram returned to normal in six months.

Myocarditis from Diphtheria

Diphtheria has commonly been known to cause myocarditis, and myocardial involvement is found in 70% of fatal cases (Gore, 1948). Three-quarters of the deaths occur on the sixth to twenty-fourth day of the illness, and in one-third of cases the manifestations appear at a time when the patient seems well. The electrocardiographic changes are either *T*-wave alterations or conduction defects (Burkhardt *et alii*, 1938).

The latter are much commoner than in other types of myocarditis. The first patient with diphtheritic myocarditis in this series was a girl, aged 13 years, who had had diphtheria for 14 days and appeared to be making satisfactory progress. She then developed marked tachycardia and rapidly progressive cardiac failure over 24 hours; her condition was complicated by a pulmonary embolus. Autopsy revealed widespread muscle-cell necrosis and patchy white-cell infiltration.

The second patient in the group was a female, aged 30 years, in whom cardiac disease was diagnosed after diphtheria at the age of eight years. Twenty-two years later an electrocardiogram still showed a left bundle-branch block and there was cardiomegaly on X-ray examination. Cardiac catheterization revealed normal hæmodynamics.

Perry (1939) has described three children in whom conduction defects persisted afterwards for many years.

Post-Operative Myocarditis

There were three patients (aged 64, 56 and 75 years) in this series in whom cardiac failure or sudden death occurred two to 11 days after operation. In one case there was a fæcal fistula without extensive sepsis, but the other two cases followed uncomplicated cataract and gall-bladder surgery. Autopsy revealed widespread white-cell infiltration of the myocardium with muscle necrosis.

Myocarditis Associated with Pregnancy

There were five cases of myocarditis associated with pregnancy—the first, second and fourth pregnancy in three cases and the third pregnancy in two cases. All were single pregnancies. Meadows (1957) states that the incidence is highest during the third pregnancy, and that twin pregnancies are more often affected than single pregnancies. The symptoms came on in the last months of pregnancy in two cases, and in one of these there was a history of an upper respiratory tract infection. In three others the symptoms occurred in the puerperium. All patients had cardiac enlargement and widespread *T*-wave changes. One patient died within a month of cardiac failure, and examination of sections of the heart showed patchy lymphocytic infiltration and muscle necrosis. Pulmonary emboli were also present. The other four patients have slowly responded to therapy. Two have had recurrent attacks of cardiac failure over three and 10 years. In one of these the

subsequent attacks were associated with a further pregnancy. Meadows (1957) has found that further pregnancies may lead to cardiac failure, but that a pregnancy may occur free from such complications.

This syndrome was first reported by Gouley *et alii* (1937), and has recently been reviewed by Rosen (1959). It consists of dyspnœa, congestive heart failure, cyanosis, sinus tachycardia, triple rhythm, pulmonary systolic murmur, a loud second sound in the pulmonary area, profuse sweating, emboli and cardiographic evidence of myocardial disease. Benchimol *et alii* (1959), reviewing 18 cases of post-partum heart failure occurring in the absence of pre-existent heart disease, recognize the following five main ætiological factors: (i) cases undoubtedly related to toxæmia of pregnancy; (ii) cases that are probably related to toxæmia of pregnancy; (iii) cases due to non-specific myocarditis; (iv) preexisting hypertensive heart disease; (v) cases of specific myocarditis (Chaga's disease and miliary tuberculosis). Fleming (1959) has noted improvement in one case of pregnancy myocarditis following the use of cortisone.

Chronic Myocarditis

There was one patient, a male, aged 18 years, who had suffered from bulbar poliomyelitis five years prior to his death. For 12 months he complained of exertional breathlessness and precordial pain, and then died suddenly. Autopsy showed gross myocardial fibrosis with normal coronary arteries. This has been grouped as a case of chronic myocarditis following acute myocarditis. Myocarditis occurs in 40% of severe cases of poliomyelitis, and may go on to fibrosis (Ludden and Edwards, 1949). It is almost a constant finding in fatal poliomyelitis (Spain *et alii*, 1950).

Parasitic Infestation

In this series there was one patient, a female, aged 40 years, who presented with precordial pain and an abnormal shadow in the region of the left ventricle. An electrocardiogram showed *T*-wave inversion. Removal of a hydatid cyst from the atrio-ventricular groove cured the patient, and the electrocardiogram returned to normal. This has previously been reported by Windsor (1954).

Classification of Myocarditis

There have been many ætiological and pathological classifications of myocarditis, but that suggested by Lustok *et alii* (1955), and based on the clinical course, seems the most satisfactory. He divides the condition into acute, recurrent and chronic (persistent). The acute type may be fulminating, subacute or benign. The benign group is further subdivided into asymptomatic (electrocardiographic changes) and symptomatic. On the whole, the electrocardiogram in this group showed localized or generalized non-specific *T*-wave changes. The extent and reversibility of the electrocardiographic changes seem to parallel the clinical course. The incidence of arrhythmias and conduction defects is usually low, but recurrent ventricular tachycardia as the chief manifestation has been described (Herman *et alii*, 1959). The outstanding gross post-mortem change was a flabby, enlarged heart. Histologically the changes were very variable. Every part of the heart may not be involved (Bowden, 1950), and several sections may be needed to demonstrate the lesion (Saphir, 1942). The inflammatory changes are usually a mixture of interstitial and parenchymatous changes (Bowden, 1950) (Figure V). The leucocytic infiltration, which is more marked in the interstitial tissues, shows a tendency to perivascular arrangement (Marcuse, 1947). The muscle may show all the degrees of change from cloudy swelling and loss of striation to complete destruction and disappearance. In some areas there may be marked vacuolation, with the sarcolemma and pyknotic muscle nuclei producing a lacework appearance. In other areas, occasional muscle fibres may remain in extensive areas of connective tissue. The cellular infiltrate may comprise variable mixtures of mononuclear cells, small round cells, polymorphs and occasionally eosinophils, phagocytic cells and giant cells. There was no correlation between the histological picture and the clinical features in the acute and subacute cases. There is considerable difference of opinion in the literature as to whether the muscle necrosis or the white-cell infiltration is the original lesion. Therapy is non-specific, but Gydell *et alii* (1955) and Garrison and Swicher (1953) have noted improvement in acute cases following the use of cortisone.

COLLAGEN DISEASE

Cases of collagen disease have been included only when the myocardial manifestations were marked or predominant. Despite the fact that it is well recognized that the heart is often involved in this group of diseases, the diagnosis is often forgotten when the dominant feature is myocardial failure. Hypersensitivity to sulphonamides (French and Weller, 1942) or to penicillin (Waugh, 1952) may cause myocarditis, or acute necrotizing vasculitis with myocardial involve-

ment the predominant feature. Erythema nodosum may also be associated with myocardial changes (Forshaw, 1959).

Polyarteritis Nodosa

There were four patients with this disorder—a woman, aged 23 years, with Raynaud's phenomena and arthritis, a woman, aged 38 years, with leg pains and fever, a woman, aged 64 years, with asthma and arthritis, and a man, aged 68 years, with vague ill health and recurrent chest infections. All presented with gross cardiac failure. Two patients had renal involvement—in fact, the man died with anuria.

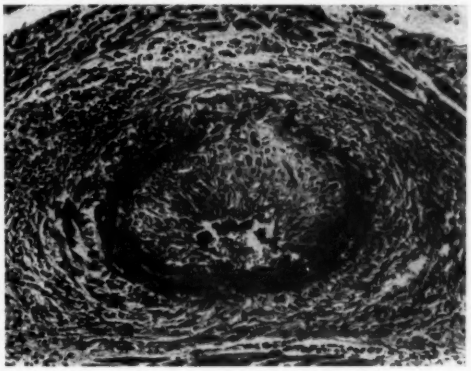


FIGURE VI

Photomicrograph of a small coronary artery showing polyarteritis nodosa. (Hæmatoxylin and eosin stain. ×180)

Diagnosis was not made in any case until autopsy. In all instances examination of the small coronary arteries showed fibrinoid degeneration of the vessel wall, white-cell infiltration round the vessel and secondary thrombosis (Figure VI). There were scattered areas of myocardial necrosis. In one case the changes in the pulmonary arteries were very extensive and may have accounted for some of the pulmonary œdema.

The heart is involved in 76% of cases of polyarteritis nodosa, and causes cardiac symptoms in 55% (Nuzum and Nuzum, 1954). Griffith and Vural (1951) found no consistent correlation between the clinical findings in the cardio-vascular system and the specific pathological changes in the cardiac organs. Malignant hypertension and myocardial infarct (Grant, 1940) or endomyocardial fibroelastosis (Watt and Lynch, 1956) may be the predominant feature.

Systemic Lupus Erythematosus

The three patients with disseminated lupus erythematosus were females, aged 38, 44 and 12 years. One had a history of rheumatoid arthritis of many years' duration and recent asthma. She presented with a myocardial infarct, and the clue to the diagnosis was splenomegaly. She has done reasonably well on cortisone and the usual measures for the control of cardiac failure.

The other patient presented with frequent ventricular premature beats and cardiac failure. Later she developed arthritis, recurrent thrombo-phlebitis and pleural effusion. Cortisone and "Chloroquine" did not cause any improvement, and she died suddenly five months after the diagnosis was made.

The girl of 12 years had anæmia and cardiac failure and did not respond in any way to cortisone therapy.

Systemic lupus erythematosus may affect the pericardium, myocardium or endocardium. The myocardium is involved by fibrinoid necrosis of the supportive structures and blood vessels, leading to secondary degeneration of the muscle fibres and focal necrosis. Occasionally, the classical picture of myocardial infarct may occur (Taubenhaus *et alii*, 1955). Congestive cardiac failure occurs in 24% of cases (Shearn and Pirofsky, 1952); but these latter authors state that arrhythmias are rare, and that premature contractions never occurred in their series. Our second case was in marked contrast to this statement.

Scleroderma

There were two patients with scleroderma.

The first was a nurse, who had had arthritis, Raynaud's phenomena, skin and facial changes and dysphagia for many years. She died suddenly after months of chronic cardiac failure. Cardiac pain associated with electrocardiographic changes occurred on several occasions. Her breathlessness was a constant problem, as it appeared to be due to a fixed thoracic cage, altered pulmonary compliance and left ventricular failure. Autopsy revealed the characteristic changes of scleroderma in the skin, œsophagus, lung and myocardium. The changes were most marked in the myocardium and took the form of patchy interstitial fibrosis. The coronary arteries were slightly atheromatous.

In contrast to this was a second patient with classical scleroderma, who for 12 months prior to his death had increasing exertional breathlessness. He died after an operation on his colon, and autopsy revealed extensive visceral pericardial thickening, with adhesions to the parietal pericardium and moderate myocardial fibrosis.

The heart is involved in 90% of cases of scleroderma (Orabona and Albano, 1958). Weiss *et alii* (1943) have stressed the difficulties of interpreting the breathlessness in these patients because of the lung and thoracic wall changes. If the myocardial interstitial fibrosis is extensive, congestive heart failure occurs, and if it is focal, conduction defects may predominate (Taubenhaus *et alii*, 1955; East and Oram,

1947). The cardiac failure may precede the cutaneous abnormality by as much as two years (Weiss *et alii*, 1943). Isolated right-heart failure may occur secondary to pulmonary hypertension (Mahrer *et alii*, 1954). Radiologically the heart is usually enlarged and may be triangular in shape, with little movement suggestive of a pericardial effusion (Barritt and O'Brien, 1952). The electrocardiogram shows low-voltage intraventricular conduction defects, a prolonged *P-R* interval and *S-T* and *T*-wave changes with occasionally auricular fibrillation (Mustakallio and Sarajas, 1954). Conduction defects have also been described (Boyd *et alii*, 1954).

METABOLIC DISORDERS

Primary Amyloidosis

The two patients with primary amyloidosis were males, aged respectively 47 and 67 years. They presented with cardiac failure, and one had a history of cardiac pain of 12 months' duration (Lazarus, 1957). This is said to be very uncommon in this disorder (Benson and Smith, 1956). Both patients had albuminuria and one had hypergammaglobulinæmia. In both the electrocardiogram showed large *Q* waves in the leads over the transitional zone, and the condition was reported as old anterior myocardial infarcts (Figure II). Examination of sections of the myocardium showed extensive homogeneous tissue forming pericellular rings and fine and coarse strands between the muscle fibres, which were partly necrotic as indicated by loss of striation, loss of nuclear staining and vacuolation of the cytoplasm (Figure VII). This tissue stained with Congo red.

The cardio-vascular system is involved in 75% of cases of primary amyloidosis (Mathews, 1954). Lindsay (1946) has emphasized the common occurrence of cardiac failure. It occurred in 56% of the series reported by Higgins and Higgins (1950). The cardiac failure is primarily due to myocardial involvement; but mechanical interference with the function of valves caused by amyloid deposits (Lindsay and Knorp, 1945), pericardial deposits, obliterative infiltration of the walls of the coronary arteries and obliterative infiltration of the walls of the pulmonary vessels leading to pulmonary hypertension may all play a part (Ballinger, 1949). The clinical picture may mimic constrictive pericarditis (Couter and Reichert, 1950). Macroglossia has been said to occur in 42% of the cardio-vascular cases (Eisen, 1946), but Baber (1947) thinks this is too high a figure. However, Benson and Smith (1956) suggest that macroglossia should be looked for in obscure heart failure, and that in its absence careful palpation of the tongue and adjacent muscles may yield useful information. Skin deposits and purpuric manifestations occur in 25% of cases (Goltz, 1952). The electrocardiographic picture is usually one of low voltage with *T*-wave changes, impaired auriculoventricular conduction and auricular fibrillation (Wessler and Freedberg, 1948). *Q* waves resembling anterior wall infarction and *P* wave *alternans* also have been described (Bernreiter, 1956).

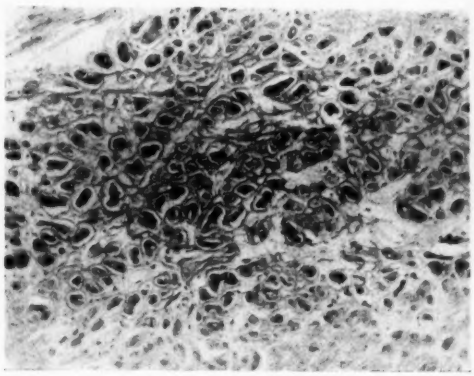


FIGURE VII

Photomicrograph of the myocardium in a case of primary amyloidosis showing pericellular deposition of amyloid. The myocardial fibres show varying degrees of degeneration. (Picro-Mallory stain. ×180)

Hæmochromatosis

The one patient with hæmochromatosis was a subnormal male, aged 31 years, who had recurrent pulmonary œdema and bronchopneumonia. He had a pulmonary systolic murmur, and an electrocardiogram showed incomplete right bundle-branch block. He was thought to have an atrial septal defect. Autopsy revealed a small septal defect, and also extensive hæmochromatosis of the myocardium. All the heart muscle had a brownish tinge, and histological examination revealed deposits of hæmosiderin throughout the myocardial fibres (Figure VIII). This pigment was most marked in the paranuclear area, but was not limited to it. In many areas the myocardial fibres appeared degenerate, and in some only sarcolemmal sheaths were present. Pigment was also present in small macrophages in the interstitial tissue, but there was no fibrosis.

Sheldon (1934) stated that myocardial failure occurred in 15% of cases of hæmochromatosis; but Bothwell *et alii* (1952) have pointed out that the average age at death is 38 years in cases of cardiac involvement, as compared with 45 to 50 years in cases without clinical evidence of any such involvement. This tendency to involve a younger age group has caused the French literature to state that there is a

syndrome complex, "*syndrome endocrino-hépato-cardiaque*", in which there are all the common manifestations of hæmochromatosis plus heart failure, and that this occurs almost exclusively in young adults (de Gennes *et alii*, 1936). In some cases the signs and symptoms of hæmochromatosis are missing or poorly developed, and the patient may present with obscure cardiac failure (Lewis, 1954). The average duration of survival after the onset of cardiac

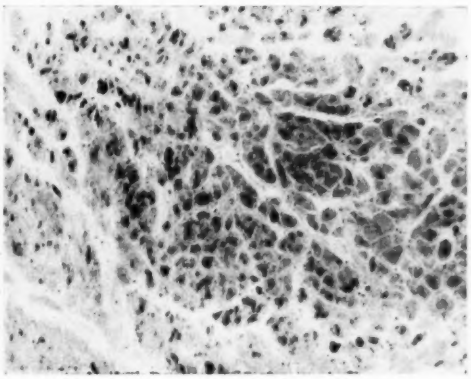


FIGURE VIII

Photomicrograph of the myocardium in a case of hæmochromatosis showing intracellular deposits of hæmosiderin. (Perl's stain. ×180)

symptoms is less than one year (Bothwell *et alii*, 1952) and may be only a few days (Bourne and Cureton, 1953). The picture is usually one of congestive cardiac failure, but cardiac pain may be a prominent feature (Swan and Dewar, 1952). Cardiac arrhythmias are frequent (Petit, 1945). There appears to be no correlation between the intensity of the hæmosiderosis, the occurrence of fibrosis, the degree of heart block and the degree of heart failure (Swan and Dewar). Radioactive iron studies by Bothwell *et alii* suggest that the heart failure is related more to the rate at which the iron is laid down in the cardiac muscle than to the absolute quantity deposited.

Alcoholic Cardiomyopathy

There were eight patients in this series with alcoholic cardiomyopathy. All were males, aged from 22 to 55 years, with a long history of alcoholism, but moderate nutrition. Œdema was the outstanding feature, and six patients presented with gross right-heart failure. In two both left-heart and right-heart failure was the presenting picture. In six a hypokinetic circulatory state was present, and in the other two there was no evidence of the classical high output state seen in beriberi. Persistent pain in the right hypochondrium was a feature in five. Tachycardia was present in five, frequent multifocal ventricular premature beats were noted in two, auricular fibrillation was present in two, and auricular flutter was present in one. The electrocardiogram usually showed *T*-wave inversion, and radiologically cardiomegaly was seen.

In four cases there was temporary improvement with diet, the exhibition of thiamine and routine therapy for heart failure, but in none was the response dramatic. Seven patients have died of chronic cardiac failure, complicated in two instances by embolic phenomena. In the seven available autopsies the heart was usually hypertrophied and dilated. Examination of sections of the myocardium in one case showed extensive fatty degeneration of the muscle fibres (Figure IX). In three others these changes were less marked, and in two the myocardial fibres almost appeared normal

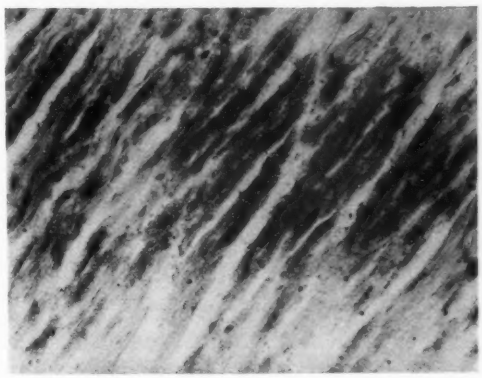


FIGURE IX

Photomicrograph of the myocardium in a case of alcoholic cardiomyopathy showing intracellular deposits of neutral fat. (Sudan IV stain. ×180)

except for some intercellular œdema. In one there were patchy areas of lymphocytic infiltration and muscle necrosis. In none was there any evidence of coronary artery disease.

Blankenhorn *et alii* (1946) have pointed out that occidental beriberi is common in alcoholics and is characterized by œdema and a normal or hypokinetic circulatory state. Only seven of his 12 patients responded to thiamine. Benchimol and Schlesinger (1953) found that only 50% of their series of alcoholics with low-output heart failure had a history of associated dietary deficiency. Cardiac failure might occur

for the first time after a period of several weeks or months of alcoholic abstinence, and the response to thiamine was variable. They stressed the frequency of tachycardia and transient gallop rhythm. Gillanders (1951) and Higginson *et alii* (1952) have described a similar clinical picture in chronic malnutrition in South Africa in Bantus. The cardiac failure may respond at first to diet, thiamine therapy and routine cardiac measures; but recurrent attacks of irreversible cardiac failure supervene, and sudden death is frequent. Evans (1959) states that the *T* waves are distinctive, and that early recognition of these changes, abstinence from drinking alcohol and treatment with digitalis will enable the patient to regain his customary health.

CARDIOMYOPATHY OF UNDETERMINED ÆTIOLOGY

The one patient in whom the diagnosis is undetermined has come to autopsy without a diagnosis being made.

The patient was a male, aged 55 years, who had recurrent cardiac failure and triple rhythm intermittently for four years. An electrocardiogram showed multifocal ventricular premature beats and *S-T* segment changes. Extensive investigation did not reveal the ætiology. At autopsy the left ventricle was dilated and hypertrophied and the left atrium dilated. There was slight atheroma of the coronary arteries without significant diminution of lumen. Examination of sections of the left ventricle showed hypertrophy of the muscle fibres and small foci of fibrosis in the inner third of the myocardium. No other significant pathological finding was present, despite the examination of multiple sections from all parts of the heart and staining with special stains.

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REFERENCES

BABER, M. D. (1947), "Amyloid Macroglossia: Report of Case", *Lancet*, **1**, 210.

BALLINGER, J. (1949), "Amyloid Heart Disease", *Amer. J. med. Sci.*, **217**, 308.

BARRITT, D. W., and O'BRIEN, W. (1952), "Heart Disease in Scleroderma", *Brit. Heart J.*, **14**, 421.

BECKER, B. J. P., CHATGIDAKIS, C. B., and VAN LINGEN, B. (1953), "Cardiovascular Collagenosis with Parietal Endocardial Thrombosis", *Circulation*, **7**, 345.

BEDFORD, D. E., and KONSTAM, G. L. S. (1946), "Heart Failure of Unknown Etiology in Africans", *Brit. Heart J.*, **8**, 236.

BENCHIMOL, A. B., CARNEIRO, R. D., and SCHLESINGER, P. (1959), "Post-Partum Heart Disease", *Brit. Heart J.*, **21**, 89.

BENCHIMOL, A. B., and SCHLESINGER, P. (1953), "Beriberi Heart Disease", *Amer. Heart J.*, **46**, 245.

BENSON, R., and SMITH, J. F. (1956), "Cardiac Amyloidosis", *Brit. Heart J.*, **18**, 529.

BERNREITER, M. (1956), "Unusual Electrocardiographic Findings in Amyloidosis", *J. Amer. Med. Ass.*, **161**, 440.

BLANKENHORN, M. A., and GALL, E. A. (1956), "Myocarditis and Myocardosis", *Circulation*, **13**, 217.

BLANKENHORN, M. A., VILTER, C. F., SCHEINKER, I. M., and AUSTIN, R. S. (1946), "Occidental Beriberi Heart Disease", *J. Amer. med. Ass.*, **131**, 717.

BOTHWELL, T. H., VAN LINGEN, B., ALPER, T., and DU PREEZ, M. L. (1952), "The Cardiac Complications of Hæmochromatosis. Report of a Case Including Radioiron Studies and a Note on Etiology", *Amer. Heart J.*, **43**, 333.

BOURNE, G., and CURETON, R. J. R. (1953), "Hæmochromatosis of the Heart", *Lancet*, **2**, 917.

BOWDEN, K. M. (1950), "Acute Myocarditis", *Med. J. Aust.*, **2**, 397.

BOYD, J. A., PATRICK, S. I., and REEVES, R. J. (1954), "Roentgen Changes Observed in Generalized Scleroderma; Report of 63 Cases", *A.M.A. Arch. intern. Med.*, **94**, 248.

BRIGDEN, W. (1957), "Uncommon Myocardial Diseases, the Non-Coronary Cardiomyopathies", *Lancet*, **2**, 1179.

BURKHARDT, E. A., EGGLESTON, C., and SMITH, L. W. (1938), "Electrocardiographic Changes and Peripheral Nerve Palsies in Toxic Diphtheria", *Amer. J. med. Sci.*, **195**, 301.

CHRISTIAN, H. A. (1950), "Clinically the Myocardium", *A.M.A. Arch. intern. Med.*, **86**, 491.

COUTER, W. T., and REICHERT, R. E. (1950), "Primary Systemic Amyloid Mimicking Chronic Constrictive Pericardial Disease", *Circulation*, **2**, 441.

DAVIES, J. W. P., and BALL, J. D. (1955), "The Pathology of Endomyocardial Fibrosis in Uganda", *Brit. Heart J.*, **17**, 337.

DE GENNES, L., DE LARUE, J., and DE VÉRICOURT, R. (1936), "*Le syndrome endocrino-hépato-cardiaque (Étude des cirrhoses pigmentaires avec infantilism et myocardiæ)*", *Presse méd.*, **44**, 377.

DEHN, H., FEIL, H., and KINDERKNECHT, K. E. (1946), "Electrocardiographic Changes in Cases of Infectious Hepatitis", *Amer. Heart J.*, **31**, 183.

DE LA CHAPELLE, C. E., and GRAEF, I. (1931), "Acute Isolated Myocarditis", *A.M.A. Arch. intern. Med.*, **47**, 942.

DENNIS, J. L., HANSEN, A. E., and CORPENING, T. N. (1953), "Endocardial Fibroelastosis", *Pediatrics*, **12**, 130.

EAST, T., and ORAM, S. (1947), "The Heart in Scleroderma", *Brit. Heart J.*, **9**, 167.

EISEN, H. N. (1946), "Primary Systemic Amyloidosis", *Amer. J. Med.*, **1**, 144.

EVANS, W. (1949), "Familial Cardiomegaly", *Brit. Heart J.*, **11**, 68.

EVANS, W. (1959), "The Electrocardiogram of Alcoholic Cardiomyopathy", *Brit. Heart J.*, **21**, 445.

EVANS, W., and WRIGHT, G. (1942), "The Electrocardiogram in Friedreich Disease", *Brit. Heart J.*, **4**, 91.

EVANS, W. F., and GRAYBIEL, A. (1946), "Electrocardiographic Evidence of Complications in Cases of Infectious Mononucleosis", *Amer. J. med. Sci.*, **211**, 220.

FIEDLER, A. (1899), "*Festschrift zur Feier des 50 Jahr bestehens des Stadtkrankenhause zu Dresden-Friedrichstadt*, **2**, 3; cited by White, P., *loco citato*.

FINE, I., BRAINERD, H., and SOKOLOW, M. (1950), "Myocarditis in Acute Infectious Disease", *Circulation*, **2**, 859.

FINLAND, M., PARKER, F., BARNES, M. W., and JOLIFFE, L. S. (1945), "Acute Myocarditis in Influenza A Infections", *Amer. J. med. Sci.*, **209**, 455.
FLEMING, H. (1959), personal communication.
FORSHAW, J. W. B. (1959), "Cardiac Involvement in Erythema Nodosum Associated with Pulmonary Tuberculosis", *Brit. Heart J.*, **21**, 142.
FRENCH, A. J., and WELLER, C. V. (1942), "Interstitial Myocarditis Following the Clinical and Experimental Use of Sulphonamide Drugs", *Amer. J. Path.*, **18**, 109.
GARRISON, R. F., and SWICHER, C. R. (1953), "Myocarditis of Unknown Etiology (Fiedler's) Treated with ACTH; Report of a Case in 7 Year Old Boy with Improvement", *J. Pediat.*, **42**, 591.
GAUNT, R. T., and LECUTIER, M. A. (1956), "Familial Cardiomegaly", *Brit. Heart J.*, **18**, 251.
GIBSON, T. C., ARNOLD, J., CRAIGE, E., and CURNEN, E. C. (1959), "Electrocardiographic Studies in Asian Influenza", *Amer. Heart J.*, **58**, 660.
GILLANDERS, A. D. (1951), "Nutritional Heart Disease", *Brit. Heart J.*, **13**, 177.
GILLIS, J. G., and WALTERS, M. B. (1954), "Acute Isolated Myocarditis Simulating Coronary Occlusion", *Amer. Heart J.*, **47**, 117.
GOLTZ, R. W. (1952), "Systematized Amyloidosis; Review of Skin and Mucous Membrane Lesions and Report of Two Cases", *Medicine*, **31**, 381.
GORE, I. (1948), "Myocardial Changes in Fatal Diphtheria", *Amer. J. med. Sci.*, **215**, 257.
GORE, I., and SAPHIR, O. (1947), "Myocarditis. A Classification of 1402 Cases", *Amer. Heart J.*, **34**, 827.
GOULEY, B. A., MCMILLAN, T. M., and BELLET, S. (1937), "Idiopathic Myocardial Degeneration Associated with Pregnancy and Especially the Puerperium", *Amer. J. med. Sci.*, **194**, 185.
GRANDELL, F. (1956), "The Heart in Progressive Muscular Dystrophy", *Amer. J. med. Sci.*, **231**, 659.
GRANT, R. T. (1940), "Observations on Periarteritis Nodosa", *Clin. Sci.*, **4**, 245.
GRIFFITH, G. C., and VURAL, I. L. (1951), "Polyarteritis Nodosa", *Circulation*, **3**, 481.
GYDELL, K., BJORCH, G., and WINKLAD, S. (1955), "Acute Fatal Myocarditis", *Acta med. scand.*, **151**, 1.
HERMAN, R. H., SCRIPTER, L. J., and MATTINGLY, T. W. (1959), "Recurrent Ventricular Tachycardia as the Chief Manifestation of Myocarditis of Unknown Etiology", *Amer. Heart J.*, **57**, 829.
HETZEL, P. S., WOOD, E. H., and BURCHELL, H. B. (1953), "Pressure Pulses in the Right Side of the Heart in a Case of Amyloid Disease and in a Case of Idiopathic Heart Failure Simulating Constrictive Pericarditis", *Proc. Mayo Clin.*, **28**, 107.
HIGGINS, W. H., and HIGGINS, W. H., JR (1950), "Primary Amyloidosis; Clinical and Pathological Study", *Amer. J. med. Sci.*, **210**, 620.
HIGGINSON, J., GILLANDERS, A. D., and MURRAY, J. F. (1952), "The Heart in Chronic Malnutrition", *Brit. Heart J.*, **14**, 213.
JAVETT, S. N., HEYMANN, S., MUNDEL, B., PEPLER, W. J., LURIE, H. I., GEAR, J., MEASROCH, V., and KIRSCH, Z. (1956), "Myocarditis in the Newborn Infant", *J. Pediat.*, **48**, 1.
JOHNSON, F. R. (1952), "Anoxia as Cause of Endocardial Fibroelastosis in Infancy", *A.M.A. Arch. Path.*, **54**, 237.
KILPATRICK, J. A., and CAUGHEY, J. E. (1955), "Changes in the Heart in Dystrophia Myotonica", AUST. ANN. MED., **4**, 200.
LAZARUS, L. (1957), "Primary Systemic Amyoidosis" AUST. ANN. MED., **6**, 116.
LEVY, R. L., and VON GLAHN, W. C. (1944), "Cardiac Hypertrophy of Unknown Cause; Study of Clinical and Pathological Features in Ten Adults", *Amer. Heart J.*, **28**, 714.
LEWIS, H. P. (1954), "Cardiac Involvement in Hæmochromatosis", *Amer. J. med. Sci.*, **227**, 544.
LINDSAY, S. (1946), "The Heart in Primary Systemic Amyloidosis", *Amer. Heart J.*, **32**, 419.
LINDSAY, S., and KNORP, W. F. (1945), "Primary Systemic Amyloidosis", *A.M.A. Arch. Path.*, **39**, 315.
LISAN, P., IMBRIGLIA, J., and LIKOFF, W. (1959), "Myocardial Disease Associated with Progressive Muscular Dystrophy", *Amer. Heart J.*, **57**, 913.
LORENZ, T. H., KURTZ, C. M., and SHAPIRO, W. H. (1950), "Cardiopathy in Friedreich's Ataxia (Spina Form of Hereditary Sclerosis)", *A.M.A. Arch. intern. Med.*, **86**, 412.
LUDDEN, T. E., and EDWARDS, J. E. (1949), "Carditis in Poliomyelitis", *Amer. J. Path.*, **25**, 357.
LUSTOK, M. J., CHASE, J., and LUBITZ, J. M. (1955), "Myocarditis", *Dis. Chest.*, **28**, 243.
MAHRER, P. R., EVANS, J. A., and STEINBERG, I. (1954), "Scleroderma; Relation of Pulmonary Changes to Œsophageal Disease", *Ann. intern. Med.*, **40**, 92.
MARCUSE, P. M. (1947), "Nonspecific Myocarditis", *A.M.A. Arch. Path.*, **43**, 602.
MATHEWS, W. H. (1954), "Primary Systemic Amyloidosis", *Amer. J. med. Sci.*, **228**, 317.
MEADOWS, W. R. (1957), "Idiopathic Myocardial Failure in the Last Trimester of Pregnancy and the Puerperium", *Circulation*, **15**, 903.
MORITZ, A. R., and ZAMCHECK, N. (1946), "Sudden and Unexpected Deaths of Young Soldiers; Diseases Responsible for Such Deaths During World War II", *A.M.A. Arch. Path.*, **42**, 459.
MUSTAKALLIO, K. K., and SARAJAS, H. S. S. (1954), "Some Aspects of Scleroderma Heart Disease", *Amer. Heart J.*, **47**, 437.
NUZUM, J. W., JR, and NUZUM, J. W., SR (1954), "Polyarteritis Nodosa", *A.M.A. Arch. intern. Med.*, **94**, 942.
O'BRIEN, W. (1954), "Endocardial Fibrosis in the Sudan", *Brit. med. J.*, **2**, 899.
ORABONA, M. L., and ALBANO, O. (1958), "Systemic Progressive Sclerosis (or Visceral Scleroderma)", *Acta. med. scand.*, Suppl. 333.
PACHOMOV, N., and CAUGHEY, J. E. (1958), "Dystrophia Myotonica—Death Under Pentothal Anæsthesia", AUST. ANN. MED., **7**, 159.
PANKE, W., and ROTTINO, A. (1955), "Endocardial Fibroelastosis Occurring in the Adult", *Amer. Heart J.*, **49**, 89.
PERRY, C. B. (1939), "Persistent Conduction Defects Following Diphtheria", *Brit. Heart J.*, **1**, 111.
PETIT, D. W. (1945), "Hæmochromatosis with Complete Heart Block", *Amer. Heart J.*, **29**, 253.
PRIOR, J. T., and WYATT, T. C. (1950), "Endocardial Fibroelastosis. A Study of 8 Cases", *Amer. J. Path.*, **26**, 969.
ROSEN, S. M. (1959), "Puerperal Cardiomyopathy", *Brit. med. J.*, **2**, 5.
RUBIN, I. L., and BUCHBERG, A. S. (1952), "The Heart in Progressive Muscular Dystrophy", *Amer. Heart J.*, **43**, 161.
RUSSEL, D. S. (1946), "Myocarditis in Friedreich's Ataxia", *J. Path. Bact.* **58**, 739.
SANDBERG, A. A., HECHT, H. H., and TYLER, F. H. (1952), "The Heart in Muscular Dystrophy", *Amer. J. Med.*, **8**, 498.
SAPHIR, O. (1942), "Myocarditis", *A.M.A. Arch. Path.*, **33**, 88.

SAPHIR, O. (1951), "Myocarditis, a General Review with an Analysis of 240 Cases", *A.M.A. Arch. Path.*, **32**, 1000.

SHEARN, M. A., and PIROFSKY, B. (1952), "Disseminated Lupus Erythematosus", *A.M.A. Arch. intern. Med.*, **90**, 790.

SHELDON, J. H. (1934), "Hæmochromatosis (Bradshaw Lecture)", *Lancet*, **2**, 1031.

SPAIN, D. M., BRADESS, V. A., and PARSONNET, V. (1950), "Myocarditis in Poliomyelitis", *Amer. Heart J.*, **40**, 336.

SWAN, W. G. A., and DEWAR, H. A. (1952), "The Heart in Hæmochromatosis", *Brit. Heart J.*, **14**, 117.

TAUBENHAUS, M., EISENSTEIN, B., and PICK, A. (1955), "Cardiovascular Manifestations of Collagen Diseases", *Circulation*, **12**, 903.

TEARE, D. (1958), "Asymmetrical Hypertrophy of the Heart in Young Adults", *Brit. Heart J.*, **20**, 1.

THOMAS, W. A., RANDALL, R. V., BLAND, E. F., and CASTLEMAN, B. (1954), "Endocardial Fibroelastosis; A Factor in Heart Disease of Obscure Ætiology", *New Engl. J. Med.*, **251**, 327.

WATT, J., and LYNCH, J. B. (1956), "Periarteritis Nodosa. Its Relationship to Endomyocardial Sclerosis", *Lancet*, **1**, 658.

WAUGH, D. (1952), "Myocarditis, Arteritis and Focal Hepatic, Splenic and Renal Granulomas Apparently due to Penicillin Sensitivity", *Amer. J. Path.*, **28**, 437.

WEISENFELD, S., and MESSINGER, W. J. (1952), "Cardiac Involvement in Progressive Muscular Dystrophy", *Amer. Heart J.*, **43**, 170.

WEISS, S., STEAD, E. A., WARREN, J. V., and BAILEY, O. T. (1943), "Scleroderma Heart Disease", *A.M.A. Arch. intern. Med.*, **71**, 749.

WESSLER, S., and FREEDBERG, A. A. (1948), "Cardiac Amyloidosis—Electrocardiographic and Pathologic Observations", *A.M.A. Arch. intern. Med.*, **82**, 63.

WHITE, P. D. (1951), "Heart Disease", 4th Edition, New York.

WHITE, P. D., and FENNEL, R. H. (1954), "Endocardial Fibroelastosis in a Man Who Died at the Age of 71", *Ann. intern. Med.*, **41**, 333.

WINDSOR, H. M. (1954), "Primary Cardiac Hydatid Disease", *Brit. J. Surg.*, **41**, 3.

WOOD, P. (1956), "Diseases of the Heart and Circulation", 2nd Edition, Eyre & Spottiswoode.

ZATUCHNI, J., AEGERTER, E. E., MOLTHAN, L., and SHUMAN, C. R. (1951), "The Heart in Progressive Muscular Dystrophy", *Circulation*, **3**, 846.

ACUTE DIFFUSE INTERSTITIAL PULMONARY FIBROSIS IN SCLERODERMA[1]

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SUMMARY

A case of generalized scleroderma with pulmonary manifestations is described.

Completely normal findings on detailed studies of respiratory function three and a half months before the patient's death allow the pulmonary lesions to be described as acute diffuse interstitial pulmonary fibrosis.

The histological appearances in the lungs are believed to represent the earliest manifestations of "scleroderma lung" yet described.

DIFFUSE interstitial inflammation and fibrosis is commonly found in the lungs of patients suffering from scleroderma (Miller *et alii*, 1959; Mahrer *et alii*, 1954; Getzowa, 1945; Kraus, 1924). The pulmonary lesions are usually regarded as chronic on pathological grounds, and there is little or no information in the literature on their rate of onset or progression. The general implication, which is borne out by our own previous experience of "scleroderma lung", is that a given radiological appearance in the lungs may be associated with all degrees of respiratory disability from minimal to severe, and that the rate of progression of respiratory disability is slow.

We report here the history of a patient with generalized scleroderma, in whom it was demonstrated that interstitial pulmonary inflammation and fibrosis developed acutely.

Mrs. J. L., then aged 41 years, first noted in May, 1958, that her hands, feet and face were "puffy". This puffiness lessened during the winter months of 1958, but returned in October. It again subsided in January, 1959, leaving the affected areas of skin thickened, tense, and "numb". During the winter of 1958, she had a number of episodes of Raynaud's phenomenon. From October, 1958, she noticed some dyspnœa on exertion. In the early months of 1959, she experienced some sensations of food "sticking" behind the sternum, and occasionally she regurgitated unaltered food and fluids immediately after eating. Her past health and family history were non-contributory. She had smoked 30 cigarettes per day until 1955.

The patient was admitted to the Royal Prince Alfred Hospital on March 26, 1959. Present over the greater part of the body surface, and most marked over the face, neck and extremities, were the skin changes she had described. The skin was dry, cool, thickened and tense; on flexion of the fingers, the abnormal skin on the digits became extremely pale. No abnormalities were found on clinical examination of the heart, lungs, abdomen or nervous system. Chemical examination of the urine showed no abnormality.

Laboratory examinations gave the following results. The hæmoglobin value and leucocyte count were normal. The blood urea content was 28 mg. per 100 ml. The total serum protein content was 6·5 grammes per 100 ml., and no significant abnormality of any fraction was detected by paper electrophoresis. An electrocardiogram showed low voltage complexes, but was otherwise within normal limits. X-ray examination with a barium bolus demonstrated œsophageal changes consistent with involvement by scleroderma. The chest X-ray findings were normal.

Because of her complaint of breathlessness in the presence of a normal chest X-ray picture, detailed studies of pulmonary function were performed on April 13.

The diffusing capacity of the lungs ($D_{L_{CO}}$) was determined by the method of Filley *et alii* (1954), as modified by Holland and Blacket (1958). Blood-gas concentrations were measured by the methods of Van Slyke and Neill (1924), and the pH of whole blood was determined anaerobically in a Stadie cell with a glass electrode and a specially calibrated Cambridge pH meter. Lung volumes were determined with a six-litre spirometer, the functional residual capacity being measured by the closed-circuit helium dilution technique. Maximum voluntary ventilation (M.V.V.) was determined over 12 seconds' breathing on the spirometer. The values of lung volumes and M.V.V. have been compared with the values predicted for the patient by the formulæ of Needham *et alii* (1954).

Lung compliance was measured during spontaneous breathing with the patient sitting erect. Intra-œsophageal pressure was measured with a thin-walled

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" Latex " balloon, 12 cm. long, containing 1 ml. of air, in the lower third of the œsophagus (Mead *et alii*, 1955). Intraœsophageal pressure, air flow rate (from a Lilly pneumotachometer) and tidal volume (by electrical integration of the flow rate) were recorded simultaneously on a direct-writing recorder at a paper speed of 25 mm. per second. The volume and pressure change between points of zero flow were measured for 10 breaths and the mean was taken.

The results of these studies are shown in Tables I and II.

TABLE I

Lung Volumes, Maximum Voluntary Ventilation, and Lung Compliance

Observation	Value at R.T.P.S.[1]	Percentage of Predicted Value
Vital capacity (V.C.)	3·03 litres	117
Functional residual capacity (F.R.C.)	2·14 litres	95
Residual volume (R.V.) ..	0·86 litre	54
Total lung capacity (T.L.C.) ..	3·9 litres	94
R.V./T.L.C. ratio	0·22	Predicted <0·41
Maximum voluntary ventilation	122 litres/min.	147
Lung compliance[2]	0·127 litre/cm. water	—

[1] Room temperature and pressure, saturated with water vapour.
[2] For a volume range up to 800 ml. at a rate of 21 breaths per minute. No change in compliance for rates up to 40 breaths per minute.

TABLE II

Ventilation, Blood Gases and Diffusing Capacity

Observation	Value
Tidal volume[1]	446 ml.
Alveolar ventilation per square metre of body surface area[1]	3·83 litres per minute
Physiological dead space[1 2]	119 ml.
Dead space/tidal volume ratio[3] ..	0·266
Whole blood pH	7·51
Arterial carbon dioxide tension ..	30·1 mm. Hg
Arterial oxygen saturation	96·9%
Oxygen consumption[3]	203 ml./minute
Respiratory exchange ratio	1·0
Diffusing capacity for carbon monoxide[4]	18·6 ml./min./mm. Hg

[1] At body temperature and pressure, saturated with water vapour.
[2] Corrected for dead space of mouthpiece.
[3] At standard temperature and pressure, dry.
[4] Mean of duplicates.

Except for a reduced residual volume, the lung volumes were normal. The M.V.V. was greater than average. Lung compliance was well within the normal range of 0·09 to 0·21 (mean 0·139) litre per centimetre of water given by Frank *et alii* (1956). The increased alveolar ventilation and the low arterial carbon dioxide tension indicated a moderate hyperventilation; and since the respiratory exchange ratio and the blood pH were high, this hyperventilation probably had its onset during the test. The physiological dead space (corrected for the dead space of the mouthpiece) and the arterial oxygen saturation were normal. The DL_{CO} of 18·6 ml. per minute per millimetre of mercury was within the normal range for young adults in this laboratory of $22·2 \pm 4·1$ ml. per minute per millimetre of mercury.

She was discharged from hospital on April 22, 1959, taking 15 mg. of prednisone daily. She remained in the same (unchanged) state of health until the last week of July, 1959, when she developed a febrile illness regarded by her local practitioner as influenza, of which

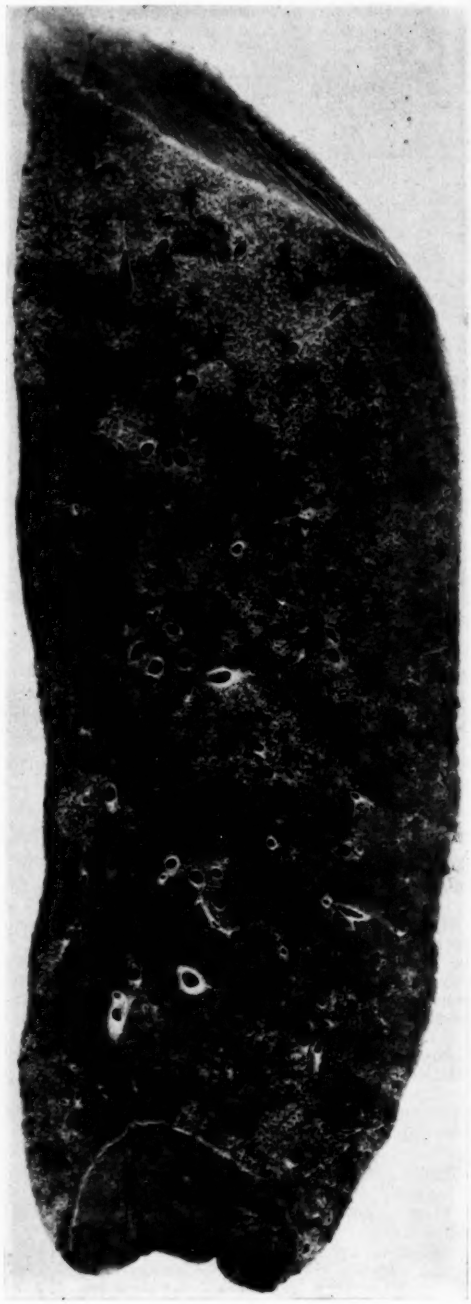


FIGURE I

Cut surface of lower lobe of left lung. For description, see text. (×1·5)

there was a mild epidemic in Sydney at the time. She was treated with chloramphenicol, and her illness did not seem in any way remarkable. On the night of August 1, she awoke very short of breath and began to cough up moderate amounts of blood.

She was admitted to Royal Prince Alfred Hospital, where she appeared severely ill. Her respirations were rapid (rate 40 per minute) and laboured, and her pulse rate was 140 per minute. She had central cyanosis, and was coughing up moderate amounts of blood-stained sputum. Her temperature was 95·6° F. Chest movement was fair; she had some coarse râles at the apices of both lungs, but no adventitious sounds at the bases, where the breath sounds were quite undiminished in loudness. The blood pressure was 180/115 mm. Hg, and there was no evidence of cardiac failure. She had a subconjunctival hæmorrhage in the right eye. The cutaneous lesions remained unaltered from the time of her previous admission.

Laboratory investigations gave the following results. The leucocyte count was 35,000 per cubic millimetre, and the sputum contained pneumococci and staphylococci. An X-ray film of the chest was reported as showing " bilateral hilar consolidation ".

Therapy was commenced with chloramphenicol and erythromycin, each in a dosage of 4·0 grammes per day. The dose of prednisone was increased to 40, and subsequently to 100 mg. per day. When oxygen was given continuously with an efficient mask, the cyanosis was greatly relieved, but the respiratory rate remained between 40 and 50 per minute. Auscultatory evidence of airway obstruction was at no time a feature of her illness. Despite these measures, the patient's condition steadily deteriorated. Her respiratory rate rose to 60 per minute, and her temperature and blood pressure fell. She died less than 36 hours after admission to hospital, and less than 48 hours after the onset of acute respiratory symptoms.

Autopsy was performed 15 hours after death. The relevant findings were as follows.

The right pleural cavity contained about 1000 ml. of clear yellow fluid, and the left pleural cavity about 500 ml. The lungs were heavy (right 725 grammes, left 660 grammes) and unusually firm. On their cut surfaces, there were pale-grey, airless areas of irregular shape up to 2 cm. in length. They were bigger and more numerous in the middle and lower lobes (Figure I). The rest of the lung tissue varied in colour from red to grey. In some areas the alveolar walls were obviously thickened, but no cysts were found.

The pericardial cavity contained 200 ml. of clear yellow fluid. The heart weighed 400 grammes; the left ventricle was moderately hypertrophied, but the right ventricular wall was of normal thickness.

The kidneys (right 135 grammes, left 165 grammes) had congested and coarsely granular surfaces. On the cut surfaces the cortex was narrowed and pale, with some red mottling.

In the brain there was a small superficial hæmorrhage over the right parietal lobe, and two small areas of hæmorrhagic softening, 2 to 3 mm. in diameter, were found in the pons.

There was no macroscopically evident abnormality in the entire gastro-intestinal tract, liver, spleen, pancreas or internal genitalia.

Microscopic examination gave the following findings. A section of the skin showed advanced changes of scleroderma. The dermis was thickened, the rete ridges of the epidermis were lost, sebaceous glands and hair follicles were absent and the sweat glands were embedded in fibrous tissue.

In the kidney, there was patchy necrosis of many glomeruli, and a few were completely necrotic. Fibrinoid necrosis had occurred in many arterioles. There was at least one recent infarct. Fibrinoid necrosis of arterioles was also found in the lower end of the œsophagus, in the pancreas, and related to the small pontine softenings.

In all parts of the lungs, alveolar walls were thickened to a varying extent by young, very cellular fibrous tissue (Figure II). The fibroblast nuclei were plump and there was little collagen present, suggesting that the fibrosis was a recent and active process. In those areas where the alveolar walls were thickest, cuboidal lining cells were prominent. Many macrophages lay

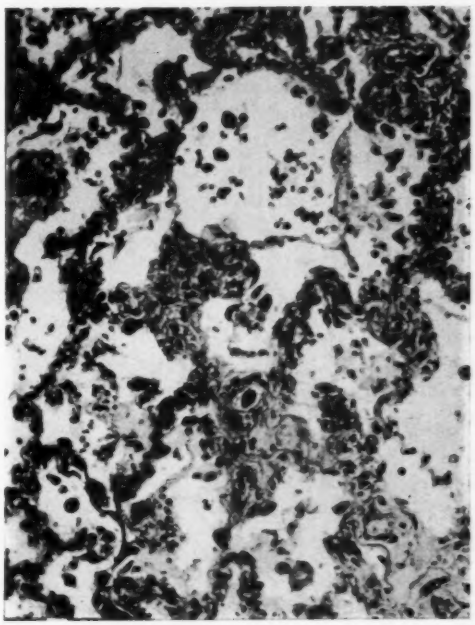


FIGURE II

Section of lung, showing infiltration of alveolar walls with plump fibroblasts and mononuclear cells. A few mononuclear cells lie free within the alveolar spaces. (Hæmatoxylin and eosin stain. ×100)

free in the alveoli. There was no fibrinoid necrosis of pulmonary vessels. Silver staining (Figure III) demonstrated a marked increase of the reticulin content of the alveolar walls.

In some areas of the lung there was a superimposed acute pneumonia, with an exudate of fibrin and polymorphonuclear leucocytes. In the lumen of some alveoli this exudate was beginning to organize. These changes occurred in the regions noted to be grey and airless on macroscopic examination.

DISCUSSION

There is no doubt on clinical and pathological grounds that this patient was suffering from generalized scleroderma. Clinically the skin lesions were characteristic, and their nature was confirmed by the diagnostic histological appear-

ances seen *post mortem*. The histological changes in the kidney and in arterioles throughout the body are uncommon but well documented in the literature. No pathological lesion of the œsophagus was seen to account for the apparently classical appearances on X-ray examination with a barium bolus. The final illness seems to have been acute post-influenzal pneumonia, in a woman who already had considerable diffuse interstitial pulmonary fibrosis. We may regard the acute pneumonia as the immediate cause of death; but the interstitial pulmonary lesion calls for more comment.

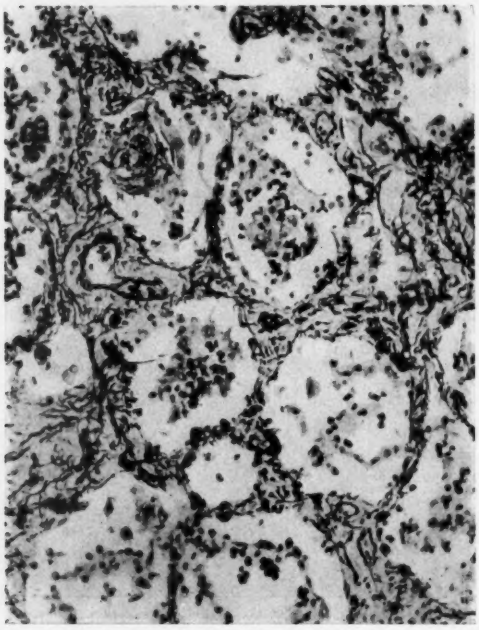


FIGURE III

Section of lung, showing increased amounts of reticulin in the alveolar walls. (Silver stain. ×100)

It had obviously been present, on pathological grounds, for more than the week during which the patient suffered from "influenza"; but it is equally apparent that interstitial fibrosis of this severity was not present three and a half months earlier, when detailed studies of pulmonary function were made.

Reports of the results of pulmonary function studies in scleroderma are not numerous (Baldwin *et alii*, 1949; Spain and Thomas, 1950; Salomon *et alii*, 1955; Miller *et alii*, 1959). The available reports indicate a pattern of reduced lung volumes, normal ventilatory capacity and arterial hypoxæmia in well-established cases. We can find no reference to studies of diffusing capacity or lung compliance in this condition; but it could confidently be expected that these would be reduced, and would provide a more sensitive index of the presence of interstitial pulmonary disease than those tests reported by earlier authors. Reduced lung compliance and reduced diffusing capacity have indeed been demonstrated in this laboratory in patients with established "scleroderma lung" (Colebatch, unpublished observations).

In fact, though the patient had complained of some dyspnœa at the end of 1958, the normal or better-than-normal values obtained for lung volumes, ventilatory capacity, diffusing capacity and lung compliance in April, 1959, make it unlikely that there was any significant lung damage present at that time. The possibility of error in the functional findings is eliminated by the constancy of the trend in the results—high lung volumes, high maximum breathing capacity, high diffusing capacity, and high compliance — all pointing away from any significant interstitial lesion. Had it been possible to repeat the functional studies in the presence of the interstitial lesion demonstrated at autopsy (and in the absence of the acute pneumonia), we are confident from our studies on other patients with interstitial disease that gross abnormalities would have been demonstrable. We may thus regard the interstitial pulmonary fibrosis as truly "acute", in the sense that it developed and became severe in a period of some three months.

This case is unique among reported cases of interstitial pulmonary fibrosis, in that it is the only one in which detailed studies of lung function showing normal results are available within a short time of death. Even in the classically "acute" interstitial fibrosis of Hamman and Rich (1944), this situation has not arisen. Indeed, it has been postulated that the acute onset and progression of the Hamman-Rich syndrome are more apparent than real, and are due to rapid progression of symptoms once respiratory reserves have been exhausted by a more chronic process. In the present case we have good grounds for using the designation "acute diffuse interstitial pulmonary fibrosis".

The post-mortem appearances in the lungs support this functional evidence. Getzowa (1944), in a detailed study, reported that the pulmonary lesions in scleroderma fell into two groups—heavy fibrosis of the alveolar walls and the development of areas of "cystic sclerosis". Cystic areas, usually regarded as indicating a long-standing process, are very common in the

lungs in this disease, but were absent in half the cases reported by Mahrer *et alii* (1954). None was found in the present case. The interstitial fibrosis itself is usually described as chronic, Mahrer *et alii* being representative in using the adjectives "thickened" and "sclerosed" to describe the alveolar walls. All the illustrations available in the literature show heavy deposition of collagen and a relative paucity of cells in the alveolar septa. Kraus (1924) is perhaps alone in referring to interstitial cellular infiltration in an earlier phase of the process.

The histological appearances in the lungs in the present case are probably the earliest yet reported. There was considerable infiltration with mononuclear cells and plump fibroblasts with deposition of increased amounts of reticulin in the alveolar walls, but collagen was not conspicuous. These appearances are commonly seen in the earlier stages of other diffuse interstitial pulmonary fibroses, such as the Hamman-Rich syndrome. Only because the patient died of an intercurrent illness was material at this stage of development obtained in the present instance. Presumably, had she lived, the changes would have progressed over a period of years to the chronic end-stage we are accustomed to see in cases of scleroderma.

The pathological changes, then, support the evidence of the functional assessment that this was an example of "scleroderma lung" of recent development. Read (1958) has postulated that the interstitial pulmonary lesions of diseases of immune origin follow a common pathological pathway, and that the histological appearances in the lungs in any individual patient or any individual disease will depend on the duration and acuteness of the process. Scleroderma is regarded by many as a disease process based on immune mechanisms; but the reported pulmonary lesions have all consisted of a chronic collagenous interstitial fibrosis. The demonstration in the present case of the early stage of the process lends support to Read's hypothesis that the classical lesions of "scleroderma lung" are the end result of the earlier lesions seen in other immune diseases.

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REFERENCES

BALDWIN, E. DE F., COURNAND, A., and RICHARDS, D. W. (1949), "Pulmonary Insufficiency", *Medicine*, **28**, 1.

FILLEY, G. F., MACINTOSH, D. J., and WRIGHT, G. W. (1954), "Carbon Monoxide Uptake and Pulmonary Diffusing Capacity in Normal Subjects at Rest and During Exercise", *J. clin. Invest.*, **33**, 530.

FRANK, N. R., MEAD, J., SIEBENS, A. A., and STOREY, C. F. (1956), "Measurement of Pulmonary Compliance in Seventy Healthy Young Adults", *J. appl. Physiol.*, **9**, 38.

GETZOWA, S. (1945), "Cystic and Compact Pulmonary Sclerosis in Progressive Scleroderma", *A.M.A. Arch. Path.*, **40**, 99.

HAMMAN, L., and RICH, A. R. (1944), "Acute Diffuse Interstitial Fibrosis of the Lungs", *Bull. Johns Hopk. Hosp.*, **74**, 177.

HOLLAND, R. A. B., and BLACKET, R. B. (1958), "Carbon Monoxide Diffusing Capacity of the Lung in Normal Subjects", AUST. ANN. MED., **7**, 192.

KRAUS, E. J. (1924), "*Zur Pathogenese der diffusen Sklerodermie*", *Arch. path. Anat.*, **253**, 710.

MAHRER, P. R., EVANS, J. A., and STEINBERG, I. (1954), "Scleroderma: Relation of Pulmonary Changes to Œsophageal Disease", *Ann. Intern. Med.*, **40**, 92.

MEAD, J., MCILROY, M. B., SELVERSTONE, N. J., and KRIETE, B. C. (1955), "Measurement of Intra-œsophageal Pressure", *J. appl. Physiol.*, **7**, 491.

MILLER, R. D., FOWLER, W. S., and HELMHOLZ, F. H. (1959), "Scleroderma of the Lungs", *Proc. Mayo Clin.*, **34**, 66.

NEEDHAM, C. D., ROGAN, M. C., and MCDONALD, I. (1954), "Normal Standards for Lung Volumes, Intrapulmonary Gas Mixing, and Maximum Breathing Capacity", *Thorax*, **9**, 313.

READ, J. (1958), "The Pathogenesis of the Hamman-Rich Syndrome", *Amer. Rev. Tuberc.*, **78**, 353.

SALOMON, A., APPEL, B., DOUGHERTY, E. F., HERSCHFUS, J. A., and SEGAL, M. S. (1955), "Scleroderma. Pulmonary and Skin Studies Before and After Treatment with Cortisone", *A.M.A. Arch. intern. Med.*, **95**, 103.

SPAIN, D. M., and THOMAS, A. G. (1950), "The Pulmonary Manifestations of Scleroderma: An Anatomic-Physiological Correlation", *Ann. Intern. Med.*, **32**, 152.

VAN SLYKE, D. D., and NEILL, J. M. (1924), "The Determination of Gases in Blood and Other Solutions by Vacuum Extraction and Manometric Measurement", *J. biol. Chem.*, **61**, 523.

URINARY AMINO-ACID EXCRETION IN HEALTH AND DISEASE[1]

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SUMMARY

Urinary amino-acid excretion was studied by paper chromatography in 208 normal and 78 abnormal subjects.

Non-specific amino-aciduria, defined as the excretion of a total amount of amino-acids exceeding 30 units on the scale described, was found in cases of Wilson's disease and hepatic coma and in one mentally retarded child. Non-specific amino-aciduria as an isolated finding is of little diagnostic value, but in conjunction with clinical data may be helpful.

Specific amino-aciduria, the excretion of one or more specific amino-acids in abnormal amounts although the total amino-acid excretion may still be normal, was found in cases of cystinosis, cystinuria, phenylketonuria and beta-amino-isobutyric "secretion". The pattern of a specific amino-aciduria is diagnostic.

Chromatography has its greatest clinical application in diagnosis in the specific amino-acidurias and in treatment in phenylketonuria, and has little or no application in prognosis at the present time, apart from helping diagnosis.

THE urinary excretion of amino-acids in human subjects was investigated originally by Dent (1946) and Wolff (1951) using paper chromatography. By this method these authors were able to give a qualitative or semi-quantitative description of the patterns of excretion of various amino-acids in the urine of normal persons and of patients suffering from various diseases. The more accurate quantitative technique of ion-exchange column chromatography has now been developed, notably by Stein (1953), but is time-consuming, and its greater precision is not often necessary for routine hospital use. Paper chromatography therefore has a place in clinical medicine, and the purpose of this report is to assess its application as a help to diagnosis, treatment and prognosis.

METHODS AND MATERIAL

Specimens of urine were collected from normal male and female adult subjects, from children and from patients suffering from various diseases. The urine samples were single specimens gathered without regard to food intake. No preservatives were added, and the urine was stored overnight in a refrigerator before use next day. The 47 normal male subjects and 59 normal female subjects were orthopædic out-patients. Those suffering from or suspected to be suffering from metabolic bone disease were excluded. The 102 normal children were siblings of patients attending the out-patient department of the Brisbane Children's Hospital. The pathological specimens were collected from in-patients and out-patients at the Brisbane Hospital, a large general hospital, and at the Brisbane Children's Hospital.

The descending technique of Paton and Wellby (1953) was used. Although desalted urine gives better definition and separation of the amino-acids, desalting was not carried out in the present series because of the large numbers of urine samples involved, and because it was found that the final reading of the chromatogram was unaltered by desalting. The volume, V, in millilitres of urine to be applied was derived from the urinary creatinine concentration by the use of the equation $V=kc^{-2/3}$, where k is a constant with a value of 7·2 and c is the urinary creatinine concentration in milligrammes per 100 millilitres (Wolff, 1951). This was satisfactory for adults and older children; but with younger children and infants, because of the low creatinine content of the urine, this formula necessitated the application of a large volume of urine, and the resultant amino-acid pattern was therefore of a moderately increased overall density. The equation assumes that creatinine excretion is normal, which may not be so in diseases such as muscular dystrophies.

Serum amino-acids were not investigated as a routine procedure. In cases of special interest, serum was deproteinized by the addition of an equal volume of acetone, and after centrifugation 120 microlitres of the supernatant were applied to the chromatogram.

After the ethanol run, the papers were air-dried. After the phenol run, the papers were oven-dried at 40° C. ± 1° C. Significant losses of amino-acids were found to occur if the temperature exceeded 40° C., as has been noted by Brush *et alii* (1951). The paper was sprayed with ninhydrin solution, and the amino-acids were identified by comparing the coloured spots with a "map" prepared from chromatograms of solutions of known amino-acids.

[1] Received on February 24, 1960.

[2] Pathology Registrar.

Proline, unless present in large amounts, was not identified by the methods used. Glutamic acid showed some variation in the position and shape of the spot. Usually it was present as an elongated band lying near the glycine-serine complex; at other times two spots would be found. This may have been due to interaction of glutamic acid and other amino-acids (Beck and Ebrey, 1956). The serine spot overlapped that of glycine, but was identified by the slightly different colour produced with ninhydrin.

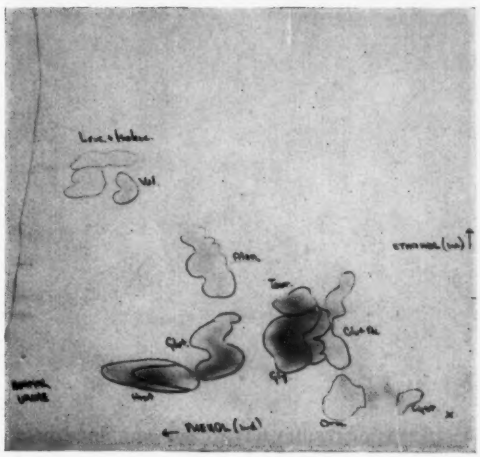


FIGURE I

Normal urinary chromatogram. Leuc., leucine; isoleuc., isoleucine; val., valine; alan., alanine; taur., taurine; gly., glycine; glut. ac., glutamic acid; glut., glutamine; hist., histidine; orn., ornithine; cyst., cysteic acid

If cystine was of special interest, it was oxidized to the more stable cysteic acid by applying hydrogen peroxide and ammonium molybdate to the urine spot before the run (Dent, 1948).

When the urine specimens were kept overnight, a thin blue band developed near the point of application of the urine to the paper. If the urine spot was treated with hydrogen peroxide and ammonium molybdate, this band failed to appear, but a more compact spot appeared in the position of cysteic acid. This band, therefore, was regarded as being due to oxidation products of cystine and was recorded as cysteic acid.

Leucine and isoleucine did not separate with the solutions used. Histidine was often present as three spots; that most distant from the point of application was green (probably methyl histidine), that nearest the point of application was bluish-purple and that intermediate between these two was reddish.

Occasionally an unidentified spot occurred under that of alanine, and has been referred to as "under alanine" (Dent, 1947).

The spot colours faded in approximately 36 hours when exposed to light and air, and very rapidly in contact with acid vapour. The chromatograms were photographed when permanent records were required.

A semi-quantitative estimation of the amount of each amino-acid present was made by visual comparison of the intensity of colour of the spot with the intensity of colour of a reference spot produced by adding 10 microlitres of 0·01M solution of glycine to the paper between the two runs. This reference spot was given an arbitrary value of "+++" (3 units) and the other spots graded from "+" (1 unit) to "+++++" (5 units). The total amino-acid excretion in each urine was obtained by addition of the excretions of each amino-acid present, as estimated by these units.

RESULTS

Normal Subjects

Normal chromatograms of urine and serum are reproduced in Figures I and II. The excretion patterns, expressed in units, of normal subjects are listed in Table I, which shows that glycine, serine, glutamine, alanine, threonine, histidine, valine, taurine (in adults), and leucine were almost invariably present. Methyl histidine, tyrosine, glutamic acid, cysteic acid, aspartic acid and ornithine were less frequently detected, and tryptophane, methionine, arginine, phenylalanine (except in children), lysine and beta-amino-isobutyric acid were only rarely seen.

The pattern of amino-acids excreted in urine was relatively constant over large numbers of subjects. Those present in greatest amounts—

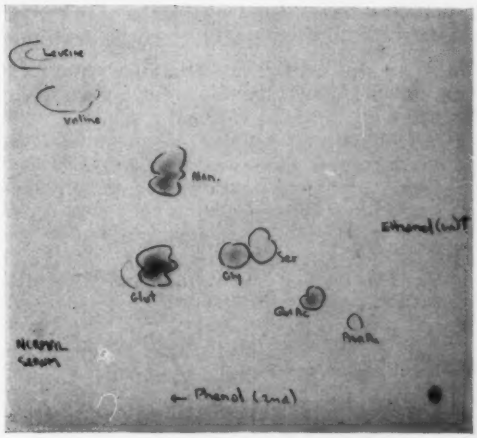


FIGURE II

Normal serum chromatogram. Asp. ac., aspartic acid; ser., serine. Other abbreviations as in Figure I

glycine, serine, glutamine, alanine, histidine and taurine—are for the most part non-essential amino-acids, which by definition the body can synthesize and hence has no need to conserve vigorously. Of the essential amino-acids, excretion of valine and leucine was constant, although of a slight degree.

However, from one individual to another the variation in the pattern of amino-acid excretion was occasionally considerable. While the amino-acids listed above were reasonably constant, one or more of them were sometimes

TABLE I

Urinary and Serum Amino-Acid Patterns in Various Disease States
(Figures in units, see text)[1]

Amino Acid	Normal		Hepatic Coma		Phenyl-pyruvia		Cystinuria	Cystinosis	Scurvy	B.A.I.B. Excretion	Pernicious Anemia	Oculo-Renal Syndrome[2]	Hartnup Disease[2]	Galactosamia[2]	Wilson's Disease
	Urine	Serum	Urine	Serum	Urine	Serum	Urine	Urine	Urine	Urine	Urine	Urine	Urine	Urine	Urine
Glycine	4	2	5	2	4	2	2	1	4	3	4	3	3	4	4
Serine	3	0–1	5	2	3	2	2	—	3	1	—	3	4	3	3
Glutamine	2	3	5	2	3	2	1	2	4	2	3	0	0	2	3
Alanine	1	2	4	2	2	3	3	3	3	1	2	2	4	4	2
Threonine	1	0–1	5	2	1	0	2	0	0	0	0	2	0	0	1
Methyl histidine	0–1	0–1	2	0–1	0	0	0	0	0	0	0	0	0	3	1
Histidine	2	0–1	3	0–1	0	0	2	—	4	2	2	1	4	3	3
Valine	1	2	4	2	1	1	0	4	4	0	0	2	4	2	Trace
Tyrosine	0–1	0–1	0–1	0–1	0	0	0	2	2	0	0	0	4	2	1
Tryptophane	0–1	0–1	3	0–1	3	0	0	0	1	2	0	0	4	0	1
Taurine	1	0	0	0	0	0	0	0	0	1	0	0	4	0	2
Leucine and isoleucine	1	0	2	0	1	0	0	4	2	1	0	2	4	1	Trace
Aspartic acid	0	1	2	2	1	0	1	1	2	0	1	0	0	1	2
Glutamic acid	1	3	1	4	2	2	0	3	2	0	2	1	4	2	1
Cysteic acid	1	1	3	0	1	1	3	2	2	0	1	1	3	2	2
Methionine	0	0	2	0	0	0	0	2	0	0	0	0	0	1	2
Arginine	0	0	0	0	0	0	4	0	0	0	0	0	0	1	0
Phenylalanine	0	0	1	0	3	3	0	2	3	0	0	0	4	2	0
Ornithine	0	0	3	0	0	0	4	2	0	0	3	0	4	0	2
Citrulline	0	0	3	0	0	0	0–1	0	0	0	0	1	0	2	0
Lysine	0	0	3	0	0	0	4	0	0	0	2	0	3	2	0
Beta-amino-isobutyric acid	0	0	0	0	0	0	0	0	0	4	0	0	0	1	0
Asparagine	0	0	2	0	0	0	0	0	0	0	0	0	4	0	0

[1] 0=not detected.
[2] For reference see text.

absent, and uncommon amino-acids were sometimes present in an otherwise normal individual. Nevertheless, repeated chromatograms showed that the particular pattern of an individual did not vary over a considerable period of time or under varying physiological conditions. Specimens of urine collected from one normal subject at varying intervals before and after meals showed no significant difference in the amino-acid patterns.

Men and women were found to excrete the same amino-acids; but women seemed to excrete more tryptophane, glutamic acid and ornithine than men. However, this could be a sampling error because of the relatively small numbers in each group. For this reason the two groups of results have been combined in a single series of 106 adult subjects.

The frequency distribution of total amino-acid excretion was calculated for the group of adults and for the group of children, and is illustrated in Figures III and IV. In each case a Gaussian distribution fitted. The mean score for adults was 19·09 units and for children 20·64 units. Estimates of the upper 2·5% points were 27·63 units for adults and 27·30 units for children. The 95% fiducial limits for the upper 2·5% points were approximately 1·5 units on either side of the estimate for adults and 1·0

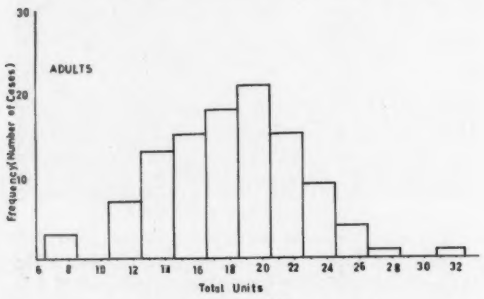


FIGURE III
Frequency distribution of total amino-acid excretion in adults

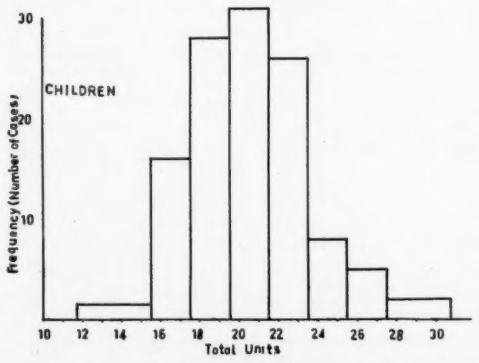


FIGURE IV
Frequency distribution of total amino-acid excretion in children

unit in the case of children. Thus 29 units would approximate the upper 2·5% point; but 30 units, which is between the 1% and 2·5% points, has been adopted. This means that a total amino-acid excretion exceeding this figure has only one chance in 40 of being normal.

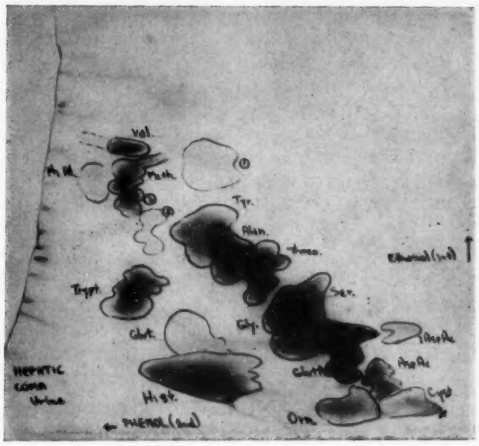


FIGURE V

Urinary chromatogram from a case of hepatic coma. Threo., threonine; ph. al., phenylalanine; tyr., tyrosine; trypt., tryptophane; 1, 2 and 3 are unknown amino-acids. Other abbreviations as in Figures I and II

Pathological Conditions

Table I illustrates the patterns of amino-acid excretion found in a number of pathological conditions.

Cystinosis.—In one case of cystinosis there was an excess of the essential amino-acids, leucine, valine, methionine and phenylalanine, although the total excretion was less than 30 units. Serum levels of valine, leucine and "underalanine" were high. The amount of cysteic acid and tyrosine in the urine was usually increased, but varied over a period of months. The urinary amino-acid patterns of the parents and one young sibling were normal.

Cystinuria.—Two patients presenting with pure cystine calculi showed a gross excess of cystine and of the basic amino-acids, ornithine, lysine and arginine. In each case the total amino-acid excretion was less than 30 units. It was not possible to study the families of these two patients.

Wilson's Disease.—One patient with Wilson's disease showed a generalized increase in amino-acid excretion, exceeding 30 units.

Lead Poisoning.—Three patients with acute lead poisoning and six patients with chronic lead nephropathy showed normal excretion patterns.

Scurvy.—Four patients with clinical scurvy showed an increase in valine, tyrosine, leucine, phenylalanine and arginine, and in each case the total excretion exceeded 30 units. In the case illustrated in Table I, the total amino-acid excretion was 34 units.

Liver Disease.—Four patients with hepatic coma were studied, and both serum and urinary amino-acids were greatly increased (Figure V). The patient listed in Table I had a total amino-acid excretion of 57 units. Chromatography demonstrated high levels of glutamic acid in the serum in all cases and low levels of arginine in the urine and serum.

A series of 19 patients who had "Q" fever and in whom there was evidence of liver involvement was investigated. Nine of the series had a total excretion greater than 30 units during the acute phase of the disease. Two of these had had previously normal chromatograms, but the remaining seven had not been examined earlier.

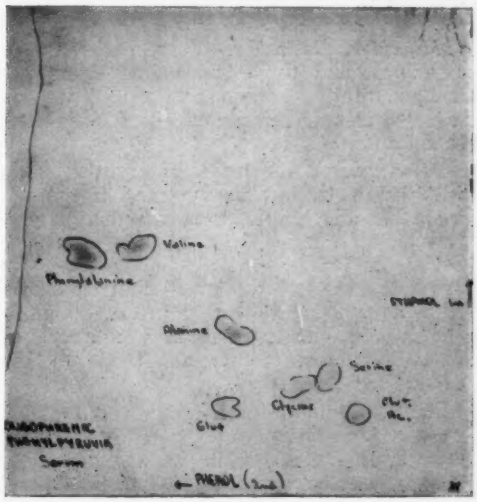


FIGURE VI

Serum chromatogram from a case of phenylketonuria

Phenylketonuria.—Three families were studied, in two of which two siblings were affected, and in the third, one of two children had the disease. The serum and urinary levels of phenylalanine were considerably raised in all cases, while the levels of the other amino-acids were normal. Once a phenylalanine-free diet was commenced, however, a generalized increase in urinary amino-acid excretion occurred, and a heavy

excretion of total amino-acids, up to 50 units, was often present. The phenylalanine level in the serum fell to 1 unit from a previous level of 3 units, and the urinary level fell, but was always excessive (2 units compared to a pre-dietary level of 5 units). As well as phenylalanine, tryptophane and methionine were in excess, and several unidentified amino-acids were present. Proline was also found in quantities large enough to be detected by the present technique. Control was maintained by daily testing of the urine with ferric chloride and by weekly chromatographic examination of serum and urine. It was found that the result of the ferric chloride test appeared to vary independently of the serum phenylalanine level.

Pernicious Anæmia.—Two patients with this disease were studied before treatment. Both had an amino-acid excretion of less than 30 units, though one showed excess ornithine and lysine.

Vitamin D-Resistant Rickets.—One patient with this condition had an increased amino-acid excretion of a generalized nature, exceeding 30 units. "Underalanine", as described in the case of Dent (1952), was present.

Muscular Dystrophy.—Four patients were examined, and three had normal qualitative and quantitative excretion patterns. The fourth excreted an unidentified amino-acid of unknown significance.

Miscellaneous.—A mentally retarded infant with cataracts, but no evidence of galactosæmia, had an amino-aciduria exceeding 30 units. One child, suffering from undiagnosed splenomegaly and mild cirrhosis of the liver, was found to excrete beta-amino-isobutyric acid in large amounts. On further investigation, the child's mother and two sisters were also found to be excretors, whereas the father did not excrete the amino-acid.

Normal amino-acid excretion was found in single cases of the following conditions: epidermolysis bullosa, pink disease, Tay-Sachs disease, hæmochromatosis, juvenile Addison's disease, nephrocalcinosis, chorea, Pierre-Robin syndrome; in two cases of idiopathic dwarfism, nephrosis, cirrhosis; in three cases of a familial demyelinating disease in the one family; and in six cases of mongolism.

DISCUSSION

There has been disagreement concerning the plasma levels of amino-acids in cystinosis. In the one case studied in this present series, there was an elevation of the serum levels of valine, leucine and "underalanine". Dent (1947) maintains that the levels are normal, and Bickel and Harris (1952) state that plasma levels are high. More recently, Clay *et alii* (1953) and Evered (1954) have confirmed the former view. The urinary pattern is distinctive, and with the clinical picture establishes the diagnosis. Chromatography is of no help in prognosis, but is eminently suitable for the investigation of siblings, in whom the amino-aciduria may precede the onset of symptoms.

Another distinctive pattern of amino-acid excretion is provided by the condition cystinuria. The results obtained agree with those of other authors. Because cystinuria is familial, it is important that not only all patients presenting with pure cystine calculi, but their families, too, should be investigated.

The amino-aciduria of Wilson's disease has been thoroughly investigated by Stein *et alii* (1954), who have shown the amino-aciduria to be of a generalized nature, as in the case in the present series. As only a very small number of cases of Wilson's disease has been described with no abnormality in amino-acid excretion, this determination is of value in diagnosis.

Wilson *et alii* (1953) have described an excess excretion of glycine, alanine, glutamine and beta-amino-isobutyric acid in acute lead poisoning. More recently, Chisolm (1957) pointed out that the amino-aciduria is of a generalized and often transitory nature and occurs simultaneously with the loss of glucose, fructose and phosphate, all of which are resorbed by the same enzymatic transfer mechanism in the renal tubular cells. He thought that lead inhibited this transfer. No samples of urine from the three cases reported here contained sugar, so that the amino-aciduria may have been missed.

Jonxis and Huisman (1954) described excess loss of threonine, serine, glycine, tyrosine, histidine, lysine and beta-amino-isobutyric acid in cases of scurvy. Vitamin C is known to be involved in the metabolism of tyrosine and phenylalanine. The excess loss of the other amino-acids points to a more generalized derangement of renal tubular resorption, because the serum levels are normal. It is doubtful whether the chromatographic findings, although spectacular, will be of much practical advantage to the clinician, for the diagnosis is essentially clinical and radiological. More investigation may reveal a usefulness in determining the duration of treatment needed to bring biochemical normality, as shown by the return to normal of the amino-aciduria and the relation of this to clinical and radiological cure. This was not carried out in the present series.

There was no clear correlation between the results of other liver function tests and the amino-aciduria present in the nine cases of

" Q " fever. The amino-aciduria present therefore is of unknown significance.

The non-specific overall increased excretion of amino-acids in hepatic coma has been found by others. Here, chromatography can give little help to the clinician. The high serum levels of glutamic acid would seem to indicate the unlikelihood of any benefit from administration of this amino-acid. On the other hand, the demonstration by Nathans *et alii* (1958) of the protection by arginine in animals against the toxic effect of high levels of ammonia, and the low levels of arginine in the urine and its absence in the serum in the cases investigated here, suggest that therapy with this amino-acid would not be illogical.

The pattern of serum amino-acids in untreated children with phenylketonuria is diagnostic. Large amounts of phenylalanine were present in the urine of affected children, even when they were taking a phenylalanine-free diet, and when the result of the ferric chloride test had become negative and serum levels of phenylalanine were low. This implies a failure of renal resorption of this amino-acid. The appearance of large amounts of tryptophane and methionine in the urine when the diet is commenced is due presumably to the presence, in the administered phenylalanine-free hydrolysate, of added DL-tryptophane and DL-methionine. The D-amino-acids, since they are not so well metabolized and are not resorbed in the renal tubules, appear in the urine. The presence of proline in significant amounts, and the presence of several unidentified amino-acids, none of which appear in the serum in detectable quantities, cannot be explained on this basis. Some defect in tubular resorption appears to accompany the artificial diet. Adequate treatment should maintain a normal serum level of phenylalanine; but until the toxic substance that determines the degree of mental defect is determined (Borek *et alii*, 1950), no logical control can be maintained. Meanwhile, chromatographic examination of the serum is a control preferable to testing the urine with ferric chloride. The siblings in an affected family should have their urine and serum examined chromatographically soon after birth.

Weaver and Neill (1954) described abnormal excretion of taurine and occasionally of lysine, cystine and leucine in a series of cases of pernicious anæmia. Todd (1959) found the 24 hours output of total alpha-amino-nitrogen to be normal in patients with vitamin B_{12} deficiency and the amino-aciduria to be mild and non-specific, although the most consistent feature was the excretion of excess taurine. The more accurate technique of column chromatography would help elucidate this question.

Although Hurley and Williams (1955) have stated that there is an increased excretion of taurine and arginine in muscular dystrophy, the four patients investigated did not show this.

The one mentally retarded infant with cataracts and generalized amino-aciduria fits no known syndrome. Isolated cases of a similar nature have been described, including a case of autism (Sutton and Read, 1958), a case of progeria (Steinberg *et alii*, 1957), and cases of congenital defects with mental retardation (Thelander and Imagawa, 1956). Probably in all cases of mental deficiency, particularly those of a progressive nature, the urinary amino-acid excretion should be investigated. The chances of positive help to the clinician are slight; but when an abnormality is found, knowledge of the causes of mental deficiency may be advanced.

In the case of the family some members of which excreted beta-amino-isobutyric acid, it appears that these subjects are " secretors " of this amino-acid. Crumpler *et alii* (1951) described apparently healthy people who excreted this substance in large amounts as an isolated anomaly. The incidence was high in familial studies, and they considered it a genetic tubular defect. It would seem, then, that the child with splenomegaly and mild cirrhosis was excreting beta-amino-isobutyric acid as a " secretor " and not because of his disease state.

No cases of " maple-syrup urine " disease (Westall *et alii*, 1957), galactosæmia (Holzel *et alii*, 1952), the oculo-cerebro-renal syndrome (Lowe *et alii*, 1952) or Hartnup disease (Baron *et alii*, 1956) were found; but the results from these authors are quoted in Table I for comparison.

Abnormalities in amino-acid excretion fall into two groups. The first group is that in which there is a generalized increase in the amino-acids excreted, so that the total value of this excretion exceeds 30 units. There is no single amino-acid or group of amino-acids constantly in excess, and this type of amino-aciduria is referred to as " non-specific ". It is found in cases of hepatic coma, Wilson's disease, galactosæmia and various isolated cases mentioned in the text. The value of 30 units is, of course, peculiar to one laboratory and is subject to many variable factors, so that each laboratory must determine its own figure. The second group is that group in which the total amino-acid excretion may be in the normal range (that is, less than 30 units), but in which there is an increase in the excretion of one or

more amino-acids. This pattern is specific to a single disease, and is referred to as specific amino-aciduria. This occurs in cystinosis, in cystinuria, in phenylketonuria, in " secretors " of beta-amino-isobutyric acid and in Hartnup disease. This classification of amino-acidurias into specific and non-specific types appears to help clinical diagnosis, because, in the case of the specific amino-acidurias, the diagnosis can be established from chromatograms of the urine alone. A specific amino-aciduria points also to a disturbance of metabolism of one or a limited number of amino-acids, whether the disturbance arises in the kidney or other sites. It supplements the classification (Dent, 1947) into renal and overflow types, which necessitates the routine taking of serum chromatograms as well as urinary chromatograms.

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REFERENCES

BARON, D. N., DENT, C. E., HARRIS, H., HART, E. W., and JEPSON, J. B. (1956), " Constant Amino-Aciduria and Other Bizarre Biochemical Features. Hereditary Pellagra-like Skin Rash with Temporary Cerebellar Ataxia ", *Lancet*, **2**, 421.

BECK, M. T., and EBREY, P. (1956), " Interaction of Substances During the Process of Paper Chromatography ", *Biochem. biophys. Acta*, **20**, 393.

BICKEL, H., and HARRIS, H. (1952), " The Genetics of Lignac-Fanconi Disease ", *Acta pædiat.* (*Stockh.*), **42**, Supp. 90, 22.

BOREK, E., BRECKNER, A., JERVIS, G. A., and WAELSCH, H. (1950), " Oligophrenia Phenylpyruvia. II. Constancy of the Metabolic Error ", *Proc. Soc. exp. Biol.* (*N.Y.*), **75**, 86.

BRUSH, M. K., BOUTWELL, R. K., BARTON, A. D., and HEIDELBERGER, C. (1951), " Destruction of Amino-acids During the Process of Paper Chromatography ", *Science*, **113**, 4.

CHISOLM, J. J., JR (1957), " Amino-aciduria as a Manifestation of Renal Tubular Injury in Lead Intoxication ", Society Transactions, Society for Pædiatric Research, *A.M.A. Amer. J. Dis. Child.*, **93**, 28.

CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. (1944), " Qualitative Analysis of Protein: A Partition Chromatographic Method Using Paper ", *Biochem. J.*, **38**, 224.

CLAY, A. D., DARMADY, E. M., and HAWKINS, M. (1953), " The Nature of the Renal Lesion in the Fanconi Syndrome ", *J. Path. Bact.*, **65**, 551.

CRUMPLER, H. R., DENT, C. E., HARRIS, H., and WESTALL, R. G. (1951), " Beta-Amino-Isobutyric Acid (Alpha-Methyl-Beta-Alanine). A New Amino-Acid Obtained from Human Urine ", *Nature*, **167**, 307.

DENT, C. E. (1946), " Detection of Amino-acids in Urine and Other Fluids ", *Lancet*, **2**, 637.

DENT, C. E. (1947), " The Amino-Aciduria in Fanconi Syndrome. A Study Making Extensive Use of Techniques Based on Paper Partition Chromatography ", *Biochem. J.*, **41**, 240.

DENT, C. E. (1948), " A Study of the Behaviour of Some Sixty Amino-Acids and Other Ninhydrin-Reacting Substances on Phenol-' Collidine ' Filter-Paper Chromatograms, with Notes as to the Occurrence of Some of Them in Biological Fluids ", *Biochem. J.*, **43**, 169.

DENT, C. E. (1952), " Rickets and Osteomalacia from Renal Tubular Defects ", *J. Bone Jt Surg.*, **346**, 266.

EVERED, D. F. (1954), " Study of Amino-acids in Biological Fluids Using Ion Exchange Resins ", Ph.D. Thesis, University of London.

HOLZEL, A., KOMROWER, G. M., and WILSON, V. K. (1952), " Amino-Aciduria in Galactosæmia ", *Brit. med. J.*, **1**, 194.

HURLEY, K. E., and WILLIAMS, R. J. (1955), " Urinary Amino-Acids, Creatinine and Phosphate in Muscular Dystrophy ", *Arch. Biochem.*, **54**, 384.

JONXIS, H. P., and HUISMAN, T. H. J. (1954), " Amino-Aciduria and Ascorbic Acid Deficiency ", *Pediatrics*, **14**, 238.

LOWE, C. U., TERREY, M., and MACLACHLAN, E. A. (1952), " Organic Aciduria, Decreased Renal Ammonia Production, Hydrophthalmos, and Mental Retardation ", *A.M.A. Amer. J. Dis. Child.*, **83**, 164.

NATHANS, D., FAHEY, J. L., and SHIP, A. G. (1958), " Sites of Origin and Removal of Blood Ammonia Formed During Glycine Infusion: Effect of L-Arginine ", *J. Lab. clin. Med.*, **51**, 124.

PATON, J. B., and WELLBY, M. L. (1953), " Analysis of Amino-Acids Present in Biological Material by Chromatographic Methods Suitable for Routine Clinical Use ", *Med. J. Aust.*, **2**, 639.

STEIN, W. H. (1953), " A Chromatographic Investigation of the Amino-Acid Constituents of Normal Urine ", *J. biol. Chem.*, **201**, 45.

STEIN, W. H., BEARM, A. G., and MOORE, S. (1954), " The Amino-Acid Content of the Blood and Urine in Wilson's Disease ", *J. clin. Invest.*, **33**, 410.

STEINBERG, A. H., SZEINBERG, A., and COHEN, B. E. (1957), " Amino-Aciduria and Hypermetabolism in Progeria ", *Arch. Dis. Childh.*, **32**, 401.

SUTTON, E. H., and READ, J. H. (1958), " Abnormal Amino-Acid Metabolism in a Case Suggesting Autism ", *A.M.A. Amer. J. Dis. Child.*, **96**, 23.

THELANDER, H. E., and IMAGAWA, R. (1956), " Amino-Aciduria, Congenital Defects and Mental Retardation ", *J. Pediat.*, **49**, 123.

TODD, D. (1959), " Observations on the Amino-Aciduria in Megaloblastic Anæmia ", *J. clin. Path.*, **12**, 238.

WEAVER, J. A., and NEILL, D. W. (1954), " Amino-Aciduria in Pernicious Anæmia and Subacute Combined Degeneration of the Cord ", *Lancet*, **1**, 1212.

WESTALL, R., DANCIS, J., and MILLER, S. (1957), " Maple Sugar Urine Disease ", *A.M.A. Amer. J. Dis. Child.*, **94**, 571.

WILSON, V. K., THOMSON, M. L., and DENT, C. E. (1953), " Amino-Aciduria in Lead Poisoning—A Case in Childhood ", *Lancet*, **2**, 66.

WOLFF, L. I. (1951), " Paper Chromatography ", *Gt. Ormond Str. J.*, **2**, 61.

THE FAILURE OF EXPERIMENTAL RENAL TUBULONECROSIS TO PRODUCE OLIGURIA IN THE RAT[1]

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SUMMARY

Acute tubulonecrosis was produced in rats by temporary clamping of the renal artery and by the subcutaneous injection of glycerol. Comparison of the mean urinary output of these animals with that of controls, both immediately after the renal damage was initiated and at 24 hours, when tubulonecrosis was clearly established, showed that there was a statistically significant increase rather than a decrease in volume. These results are interpreted as showing that tubulonecrosis itself is not an important cause of oliguria. It is concluded that it is misleading and incorrect to use the term "acute tubular necrosis" as synonymous with the acute anuria syndrome.

SINCE interest in it was revived during the War of 1939 to 1945, the acute anuria syndrome has rapidly passed through successive nosological fashions. At first, classification was based on ætiology, and such names as "transfusion kidney" (De Navasquez, 1940), "crush syndrome" (Dunn *et alii*, 1941), "traumatic anuria" (Bywaters and Dible, 1942), "blackwater fever anuria" (Maegraith, 1944) and "shock kidney" (Van Slyke, 1948) were applied. In 1946, Lucké, struck by the similarity of histological findings even though many agents were the cause, called the condition "lower nephron nephrosis", and this became a popular term for some years. However, in 1949, McManus and Rutledge stated that this was a misnomer, as the loss of function was largely in the proximal parts of the nephron, and others soon agreed (Oliver *et alii*, 1951). After this, the condition was called "acute nephrosis" by Waugh (1953) and "acute tubular nephrosis" by Moon (1953); but Bull *et alii* (1950), impressed with the severe tubular damage in some of their cases, termed the syndrome "acute tubular necrosis". This term has remained in use (Lowe, 1952; Oliver, 1953; Bull, 1956; *Lancet*, 1960), although others have remained loyal to the earlier names or have avoided the issue by referring to "acute renal failure" (Merivale, 1958; Munck, 1958).

The importance of the term "acute tubular necrosis" lies in its relation to the pathogenesis of the anuria, for it has been implied that in some way necrosis of tubules suppresses the production of urine, and that this oliguria will continue until the tubules have regenerated (Bywaters and Dible, 1942; Lucké, 1946; Van Slyke, 1948; Edwards and Whyte, 1958). However, of the many points against this view, probably the most important are, first, that in many cases of acute anuria little or no tubulonecrosis has been found (Brun and Munck, 1957; Munck, 1958; Finckh, unpublished observations), and second, that necrosis affecting both proximal and distal tubules has been recorded in patients who die of burns who have never shown oliguria (Sevitt, 1956; Graber and Sevitt, 1959). In addition, although tubulonecrosis is easily produced in animal experiments by renal artery occlusion (Scarff and Keele, 1943; Badenoch and Darmady, 1947; Koletsky, 1954; Burwell, 1955) or by exogenous agents (mercury salts—Oliver, 1953; oxalates—Dunn *et alii*, 1924; uric acid—Dunn and Polson, 1926; and subcutaneous and intraperitoneal exhibition of glycerol—Finckh, 1957 and 1959), acute anuria has rarely been observed, and then usually only when the renal damage was extremely severe.

The present paper records studies on the urinary output of rats in which moderate or severe tubulonecrosis was produced by renal artery clamping or by subcutaneous injections of glycerol. There was an increase rather than a decrease in urinary volume, both immediately after renal damage had been initiated, and also after 24 hours when tubulonecrosis was shown to be clearly established.

MATERIALS AND METHODS

Female albino rats weighing 160 to 200 grammes were used. Control studies of urinary output were made on 25 rats not used for the present experiments, and on the test rats during the two days prior to renal artery clamping or glycerol injection. The rats were kept in individual cages having coarse wire floors, and placed on individual trays. Absorbent paper of uniform thickness was placed in the trays over coarse sawdust, in order to record the time and size of speci-

[1] Received on June 23, 1960.

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mens of urine passed. The specimens of urine produced "blots" of varying size, and these were allowed to spread to their maximum and dry before they were compared with blots on a test sheet of similar paper which had been made with known volumes of urine. Drinking water was freely available to the animals throughout the experiments.

Renal Artery Clamping

Eighteen rats were subjected to right nephrectomy and then allowed to recover for three weeks. Under ether anæsthesia, the left renal artery was carefully dissected from the renal vein and completely occluded with a wire clamp constructed from a small safety-pin, with the jaws covered by fine polyethylene tubing. The clamp was removed under anæsthesia one hour later.

Subcutaneous Glycerol Injection

Injections of 50% glycerol solution in 0·85% saline were given subcutaneously into the anterior abdominal wall of 24 rats in doses of 5·3 ml. per kilogram of (anhydrous) glycerol.

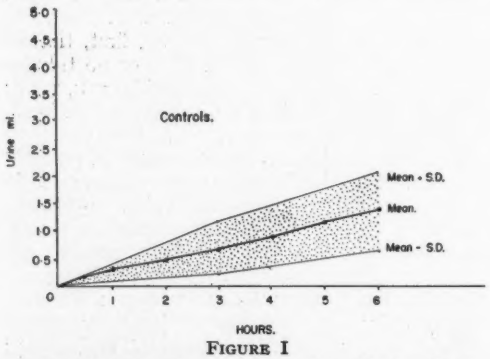


FIGURE I

Cumulative urinary output of 67 control animals, tested over six hours. Water was freely available before and during all the test periods. (S.D., standard deviation)

Histological Examination

The rats were killed 30 hours after the commencement of each experiment, and thin slices of kidney were fixed in Carnoy's fixative, paraffin embedded, sectioned and stained with hæmatoxylin and eosin.

RESULTS

Control Experiments

The urinary outputs in the control series were reasonably uniform at from 0·1 to 0·3 ml. per hour (Figure I). A group of animals was tested on five successive days, and it was found that single animals varied from day to day within fairly consistent limits, but that occasional animals had a higher reading on single days. A rise of room temperature led to some reduction in urinary output, and for this reason, and in an attempt to eliminate individual variation, the renal output of the test animals was observed on the two days prior to the experimental procedures.

Clamping of the Residual Renal Artery

After release of the renal artery clamp, there was an immediate colour change over small or larger areas of the cortex, from the dark purple of the clamped kidney to bright red.

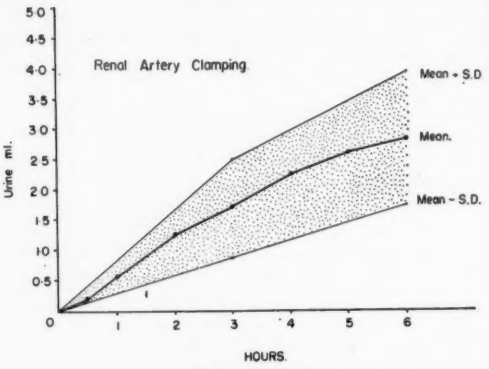


FIGURE II

Cumulative urinary output of 18 test animals (renal artery clamping for one hour). These readings were begun immediately after the release of the clamp. The urinary output over this period was significantly higher than that of the controls ($t=5·9$)

These bright-red areas became raised and slowly spread to become confluent and involve the whole kidney in five to seven minutes. The cumulative urinary outputs were measured

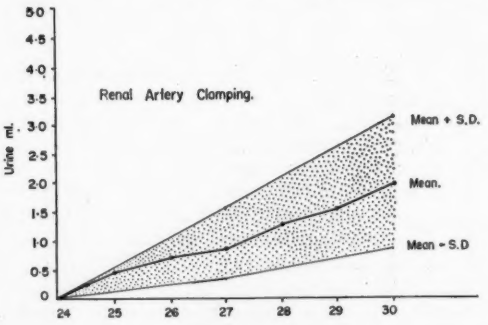


FIGURE III

Cumulative urinary output of 18 test animals (renal artery clamping). These readings were begun 24 hours after the release of the clamp, and the animals were killed immediately after the test period. The kidneys were then fixed and examined histologically, and all showed tubulonecrosis. Despite the tubulonecrosis, the urinary output over six hours was significantly higher than that of the controls ($t=2·9$)

during the six hours immediately following release of the clamp. These showed mean values which were significantly higher than those of the normal controls, the lowest figures

being within the normal range and the highest being about three times the normal volume (Figure II).

The cumulative urinary outputs were also observed for six hours on the following day, as

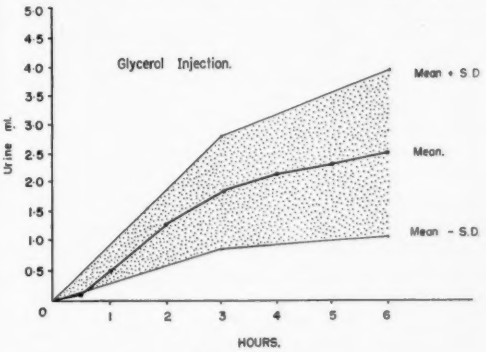


FIGURE IV

Cumulative urinary output of 24 test animals (subcutaneous glycerol injections). These readings were begun immediately after the injections. The urinary output was significantly higher than that of the controls ($t=5\cdot8$)

renal necrosis was known to be clearly established at that stage. These again showed mean urinary volumes which were significantly above normal, and some animals excreted well above their normal amounts of urine (Figure III).

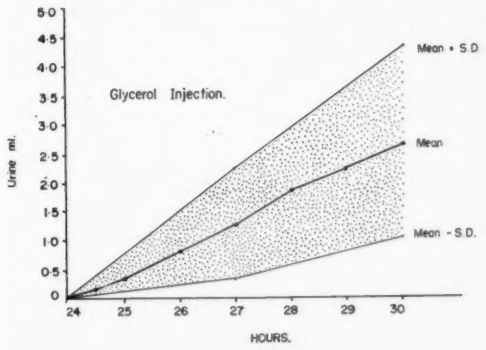


FIGURE V

Cumulative urinary output of 24 test animals (glycerol injections). These readings were begun 24 hours after the injections, and the animals were killed immediately after this test period. Histological examination of the kidneys of the animals showed tubulonecrosis in all. Despite the tubulonecrosis, the urinary output over six hours was significantly higher than that of the controls ($t=6\cdot0$)

Histological examination of the kidneys of these animals showed moderate to considerable tubulonecrosis in all (Figure VI). This affected mainly proximal convoluted tubules, and occurred in all parts of the cortex. A few cells in distal convoluted tubules and collecting tubules were also affected, but the glomeruli appeared normal. Hyaline and granular casts composed of protein or cell débris were found in the distal and collecting tubules in varying amounts, being less prominent in those animals with the higher urinary outputs.

Subcutaneous Injections of Glycerol

The subcutaneous injection of glycerol was followed by considerable diuresis, beginning soon after the injection (Figure IV). The urine was

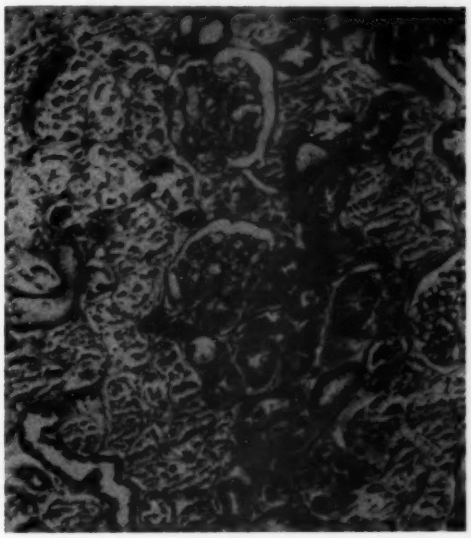


FIGURE VI

Section of renal cortex obtained 30 hours after the renal artery had been clamped for one hour. The glomeruli and some proximal and distal convoluted tubules are normal, but many other proximal convoluted tubules are composed of necrotic cell masses lying free inside the basement membranes. Some distal convoluted tubules contain dense casts and also show necrosis of cells (bottom, right of centre). (Hæmatoxylin and eosin stain. ×150)

heavily stained with hæmoglobin, owing to the rapid hæmolysis which follows these injections (Cameron and Finckh, 1956). On the next day the mean urinary volumes were also significantly higher than those of the normal controls, some being within the normal range and others being considerably higher (Figure V).

The histological lesions in the kidneys were those described previously (Finckh, 1957). There were extensive areas of proximal convoluted tubule necrosis and some necrotic

cells in distal convoluted tubules and collecting tubules (Figure VII). Hyaline and granular casts were prominent in distal and collecting tubules, especially in the animals with the lower urinary outputs.



FIGURE VII

Section of renal cortex obtained 30 hours after the subcutaneous injection of glycerol. The appearances are similar to those in Figure VI, but there is in addition diffuse hæmoglobin staining of the necrotic cells and of the casts inside the tubules. (Hæmatoxylin and eosin stain. ×150)

DISCUSSION

The course of acute renal failure has been described as having three phases, the phase of onset, the phase of oliguria and the phase of diuresis (Van Slyke, 1948; Bull, 1956). The phase of onset occurs during an episode of shock or acute poisoning, when the arterial perfusion of the kidney is considerably lowered. Anuria during this phase is generally considered to be rapidly reversible and to be caused by reduced glomerular filtration pressure. The manner in which the second phase, that of prolonged oliguria, is brought about is not clear, for it may occur despite a return of the systemic blood pressure to normal or above normal levels. It is commonly held that this phase results from renal damage from ischæmia or toxic action during the onset phase (Bull, 1956), and it has been held by many that it is damage and necrosis of the tubules which produce the oliguria (Lucké, 1946; Van Slyke, 1948; Kaplan and Fomon, 1953). Numerous workers have studied the changes in the kidneys produced by renal artery clamping, on the premiss that their findings were a model of those obtaining in humans (Scarfe and Keele, 1943; Badenoch and Darmady, 1947; Koletsky, 1954; Burwell, 1955); but these studies have not been concerned with urinary output in the animals. It is for this reason that the effects of renal artery occlusion have now been studied with particular emphasis on whether or not oliguria supervened.

The subcutaneous injection of glycerol produces immediate intravascular hæmolysis and hæmoglobinuria (Cameron and Finckh, 1956), and soon afterwards tubulonecrosis and distal cast formation can be demonstrated in the kidneys (Finckh, 1957). These changes resemble closely those described in patients who have died from anuria following incompatible blood transfusion (Bordley, 1931; Ayer and Gauld, 1942), and this method also has therefore been used to produce tubulonecrosis prior to a study of urinary output.

The histological appearances of the kidneys damaged in these ways resembled those described previously (Koletsky, 1954; Finckh, 1957). Evidence of severe damage was most pronounced in the proximal convoluted tubules, but other tubules were also affected. The glomeruli showed no significant changes. In both the artery-clamping and the glycerol-injection experiments, the lesions were distinctly patchy, with isolated groups of necrotic tubules lying between histologically normal tubules (Figures VI and VII).

The results of both sets of experiments show that considerable renal tubulonecrosis may be produced without oliguria supervening during the 30 hours after the initiation of renal damage. Not only did oliguria not occur, but when the urinary output was abnormal it was in the direction of an increase, which was often pronounced. This polyuria began within two hours of the release of the clamp or the injection of glycerol, and was present during the following day (Figures II to V). Whilst it might be argued that after the injection of glycerol there was an osmotic diuresis from red-cell breakdown products or from glycerol itself, which acted contrary to a general trend towards oliguria, this would not apply to the arterial clamp experiments. In this regard, the urinary outputs on the second day were of greatest interest, for despite the histologically distinct necrosis of tubule cells at this stage, the mean outputs were well above normal.

These results may now be viewed in the light of some current theories on the pathogenesis

of the phase of oliguria. Those who hold that tubular damage or even tubular necrosis is essential have expressed divergent views on its mechanism. Some have maintained that the damage and necrosis lead to backflow of urine from the tubules by a process of excessive reabsorption (Bywaters and Dible, 1942; Lucké, 1946; Van Slyke, 1948; Kaplan and Fomon, 1953), while others have considered that ischæmia causes rupture of tubule basement membranes (tubulorrhexis), leading to interstitial œdema of the kidneys and reduced intrarenal blood flow (Bull *et alii*, 1950; Oliver, 1953). It has also been held that in the later stages of oliguria extensive and irregular growth of tubule cells during regeneration after necrosis produces blockage in many nephrons (Oliver, 1953; Dible, 1953). This third view does not directly concern us at present, as it takes about three days for regeneration to become conspicuous in the experimental animal (Finckh, 1957), and by this time in humans the phase of oliguria has been well established.

Any theory which suggests that oliguria is the direct result of severe tubular damage is incompatible with the present findings, for it has been possible to produce moderate or even severe tubulonecrosis in the experimental animal without oliguria supervening. This is not to say that tubulonecrosis does not occur in patients with acute anuria, for it has been well documented in many cases. Functional studies in cases of this type have also indicated considerable damage to proximal convoluted tubules, as there are often glycosuria, aminoaciduria, reduced ability to reabsorb electrolytes and low PAH extraction ratios, even during the phase of oliguria (Bull, 1953, 1956). However, these abnormalities are evidence of reduced absorption, not increased absorption by the tubule cells. In the present experiments, too, the mean urinary output of the rats was increased rather than decreased, which suggests that the tubulonecrosis caused failure of absorption rather than excessive absorption.

The use of the term "acute tubular necrosis" as synonymous with "acute anuria" seems therefore to be both misleading and incorrect. Acute tubulonecrosis is a histological lesion present in some, but not all, patients with "acute anuria", and also present at times in patients who have not had oliguria. Nevertheless, although the tubulonecrosis is not the principal cause of the oliguria, the association between the two suggests that they may well be causally related.

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REFERENCES

AYER, G. D., and GAULD, A. G. (1942), "Uræmia Following Blood Transfusion", *A.M.A. Arch. Path.*, **33**, 513.

BADENOCH, A. W., and DARMADY, E. M. (1947), "The Effects of Temporary Occlusion of the Renal Artery in Rabbits and its Relationship to Traumatic Uræmia", *J. Path. Bact.* **59**, 79.

BORDLEY, J. (1931), "Reactions Following Transfusion of Blood with Urinary Suppression and Uræmia", *Arch. intern. Med.*, **47**, 288.

BRUN, C., and MUNCK, O. (1957), "Lesions of the Kidney in Acute Renal Failure Following Shock", *Lancet*, **1**, 603.

BULL, G. M. (1953), "Acute Tubular Necrosis", in "Recent Advances in Pathology", 6th Edition, edited by Hadfield, G., Churchill, London, 286.

BULL, G. M. (1956), "Osmotic Diuresis in Acute Tubular Necrosis", in "Modern Views on the Secretion of Urine", Churchill, London, 271.

BULL, G. M., JOEKES, A. M., and LOWE, K. G. (1950), "Renal Function Studies in Acute Tubular Necrosis", *Clin. Sci.*, **9**, 379.

BURWELL, R. G. (1955), "Changes in the Proximal Tubule of the Rabbit Kidney after Temporary Complete Renal Ischæmia", *J. Path. Bact.*, **70**, 387.

BYWATERS, E. G. L., and DIBLE, J. H. (1942), "The Renal Lesion in Traumatic Anuria", *J. Path. Bact.*, **54**, 111.

CAMERON, G. R., and FINCKH, E. S. (1956), "The Production of an Acute Hæmolytic Crisis by the Subcutaneous Injection of Glycerol", *J. Path. Bact.*, **71**, 165.

DE NAVASQUEZ, S. (1940), "The Excretion of Hæmoglobin with Special Reference to the 'Transfusion' Kidney", *J. Path. Bact.*, **51**, 413.

DIBLE, J. H. (1953), "The Morbid Histology of Acute Tubular Necrosis", in "Recent Advances in Pathology", 6th Edition, edited by Hadfield, G., Churchill, London, 292.

DUNN, J. S., GILLESPIE, M., and NIVEN, J. S. F. (1941), "The Renal Lesions in Two Cases of the Crush Syndrome", *Lancet*, **2**, 549.

DUNN, J. S., HAWORTH, A., and JONES, N. A. (1924), "The Pathology of Oxalate Nephritis", *J. Path. Bact.*, **27**, 299.

DUNN, J. S., and POLSON, C. J. (1926), "Experimental Uric Acid Nephritis", *J. Path. Bact.*, **29**, 337.

EDWARDS, K. D. G., and WHYTE, H. M. (1958), "Anuria, Alias Acute Renal Failure; The General Principles of Pathogenesis and Treatment", *Med. J. Aust.*, **2**, 356.

FINCKH, E. S. (1957), "Experimental Acute Tubular Nephrosis Following Subcutaneous Injection of Glycerol", *J. Path. Bact.*, **73**, 69.

FINCKH, E. S. (1959), "The Indirect Action of Subcutaneous Injections of Glycerol on the Renal Tubules in the Rat", *J. Path. Bact.*, **78**, 197.

GRABER, I. G., and SEVITT, S. (1959), "Renal Function in Burned Patients and its Relationship to Morphological Changes", *J. clin. Path.*, **12**, 25.

KAPLAN, S. A., and FOMON, S. J. (1953), "Function Recovery Pattern in Acute Renal Failure Following Ingestion of Mercuric Chloride", *A.M.A. Amer. J. Dis. Child.*, **85**, 633.

KOLETSKY, S. (1954), "Effects of Temporary Interruption of Renal Circulation in Rats", *A.M.A. Arch. Path.*, **58**, 592.

Leading Article (1960), "Acute Tubular Necrosis", *Lancet*, **1**, 371.

LOWE, K. G. (1952), "The Late Prognosis in Acute Tubular Necrosis; An Interim Follow-up Report on 14 Patients", *Lancet*, **1**, 1086.

LUCKÉ, B. (1946), "Lower Nephron Nephrosis. (The Renal Lesions of the Crush Syndrome, of Burns, Transfusions, and Other Conditions Affecting the Lower Segments of the Nephrons)", *Milit. Surg.*, **99**, 371.

MCMANUS, J. F. A., and RUTLEDGE, G. I., JR (1949), "'Lower Nephron Nephrosis', a Misnomer for the 'Crush' Kidney", *Amer. J. Path.*, **25**, 771.

MAEGRAITH, B. J. (1944), "Blackwater Fever Anuria", *Trans. roy. Soc. trop. Med. Hyg.*, **38**, 1.

MERIVALE, W. H. (1958), "Biochemical Changes in Acute Renal Failure", *Guy's Hosp. Rep.*, **107**, 426.

MOON, V. H. (1953), "Acute Tubular Nephrosis, a Complication of Shock", *Ann. Intern. Med.*, **39**, 51.

MUNCK, O. (1958), "Renal Circulation in Acute Renal Failure", Blackwell, Oxford, 1.

OLIVER, J., MCDOWELL, M., and TRACY, A. (1951), "The Pathogenesis of Acute Renal Failure Associated with Traumatic and Toxic Injury. Renal Ischæmia, Nephrotoxic Damage and the Ischemuric Episode", *J. clin. Invest.*, **30**, 1305.

OLIVER, J. (1953), "Correlations of Structure and Function and Mechanisms of Recovery in Acute Tubular Necrosis", *Amer. J. Med.*, **15**, 535.

SCARFF, R. W., and KEELE, C. A. (1943), "The Effects of Temporary Occlusion of the Renal Circulation in the Rabbit", *Brit. J. exp. Path.*, **24**, 147.

SEVITT, S. (1956*a*), "Distal Tubular Necrosis with Little or No Oliguria", *J. clin. Path.*, **9**, 12.

SEVITT, S. (1956*b*), "Distal Tubular and Proximal Tubular Necrosis in the Kidneys of Burned Patients", *J. clin. Path.*, **9**, 279.

VAN SLYKE, D. D. (1948), "The Effects of Shock on the Kidney", *Ann. intern. Med.*, **28**, 701.

WAUGH, D. (1953), "Acute Nephrosis; The Kidney of Acute Renal Failure", *Amer. J. med. Sci.*, **226**, 310.

EXPERIMENTAL CHOLANGITIS FOLLOWING BILIARY OBSTRUCTION[1]

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SUMMARY

Experiments have been performed on rats in which biliary obstruction has been created and followed by an intravenous injection of a suspension of *Bacterium coli*. Rats with obstruction only and with injection of *B. coli* only have been used as controls.

The experiments show not only that biliary obstruction predisposes to infection of the liver and bile ducts, but also that the infection is accentuated and accelerated.

The results also clearly indicate that the invading microorganisms reach the liver and bile ducts by the hæmatogenous route.

ALTHOUGH there is little doubt that in the human, biliary obstruction predisposes to the occurrence of acute cholangitis (Himsworth, 1954; Kalk, 1956) uncertainty still exists as to the route of bacterial invasion leading to the inflammation. There are two acknowledged possibilities, an ascending and a descending route. The ascending route is by the bile ducts themselves (or their concomitant lymphatics), and the descending or hæmatogeneous route is by either the hepatic artery or portal vein. A necessary postulate for the acceptance of the hæmatogenous route is that biliary obstruction in some way results in a decrease in resistance of the biliary tree to occasional blood-borne organisms, so causing it to become a *locus minoris resistentiæ* to infection with subsequent inflammation and suppuration.

The experiments to be described were undertaken to show that suppurative cholangitis can possibly, indeed is most likely, to result from hæmatogenous spread of microorganisms. The plan of the experiment was, first, to examine the incidence of spontaneous infection of the bile ducts following experimental biliary obstruction, and secondly, to investigate the effects of intravenous injection of a small dose of *Bacterium coli* into either the portal vein or the inferior vena cava. As controls, the livers of normal rats and of rats subjected to bacterial injections without biliary obstruction were examined.

No previous account has been found in the literature of experiments which have successfully produced suppurative cholangitis in animals with biliary obstruction, although Wilkie (1928) succeeded in producing chronic cholecystitis by a single intravenous injection of streptococci into rabbits with cystic duct obstruction. Umber and Heine (1924), after creating total or partial biliary obstruction, introduced organisms into both the systemic and portal venous circulations. They demonstrated damage to the central portion of the hepatic lobules when the biliary obstruction had been present for 24 hours, and leucocytic infiltration of the walls of the bile ducts after long-standing biliary stasis. Natucci (1935) injected virulent *Staphylococcus aureus* intravenously into dogs and rabbits with biliary obstruction, but in none of these animals did he find any inflammatory focus in the hepatic parenchyma.

METHOD

Production of Biliary Obstruction

Under ether anæsthesia, and with reasonable aseptic precautions, the anterior abdominal wall was incised and the liver retracted upwards. The common bile duct with its surrounding connective tissue and presumably concomitant lymphatic vessels was dissected free from the attendant artery and vein from a point just distal to the entrance into the bile duct of the last hepatic duct to a point just proximal to the entrance of the first pancreatic duct.

Two cotton ligatures were tied tightly around the bile duct and connective tissue as far apart as possible, and the whole was divided midway between the two ligatures. This double ligation and the severing of the bile duct proved to be necessary, for after single ligation only, recanalization was found to occur very frequently. This phenomenon has previously been described by Trams and Symeonidis (1957).

Preparation of Suspension of Organisms

The organism selected was a strain of *B. coli* isolated from the swollen bile duct of a rat studied for the effect of biliary obstruction alone. A colony from the stock culture was inoculated into nutrient broth overnight, and this subculture was then standardized in a photo-

[1] Received on April 19, 1960.
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meter with respect to transmittance of light. The transmittance was compared with that of sterile broth and expressed as a percentage. By this means it was possible to obtain by dilution any given concentration of organisms for similar experiments at different times. Injections of bacterial suspensions of decreasing concentrations were first carried out in the normal rat, until a dilution was reached which had only minor effects on animals without biliary obstruction when 1 ml. of the suspension was injected into the portal vein. This concentration and dose were used in all subsequent experiments. The light transmittance of the concentration selected was 98%, and this represented about a one-hundredfold dilution of the original overnight broth culture.

When intravenous injections of bacteria were performed, precautions were taken to minimize contamination at the site of injection by filling the needle with sterile broth before the actual injection.

Some intravenous injections were made into the portal vein, others into the inferior vena cava. In each case, an injection was given either at the time when biliary obstruction was created or seven days later; in the latter instance, vitamin K was given to the rats by intramuscular injection to minimize bleeding at the second operation.

Controls

The controls consisted of normal rats, and of animals that received either sterile broth or bacterial injection in the absence of biliary obstruction. A further group of controls consisted of rats which had biliary obstruction only, and of animals which both had biliary obstruction and received intravenous injections of sterile broth.

The animals can be grouped as shown in Table I.

RESULTS

Group I: Control

(*a*) *Normal Rat Liver.*—There was very little fibrous tissue in the portal tracts, usually sufficient to form only a scanty stroma surrounding the radicles of the portal vein, hepatic artery and bile ducts (Figure I). Cellular elements were confined to a few fibrocytes and lymphocytes with only an occasional polymorphonuclear leucocyte.

(*b*) *Sterile Broth Injection Only.*—In this group, five animals were killed at two, seven and 14 days. Injections into the portal veins of these rats without obstruction caused no significant variation from normal.

(*c*) *B. Coli Injection Only.*—Five animals were killed two days after the injection of organisms and five after seven days. The only abnormality found was an increase of inflammatory cells, including a few polymorphs in the portal tracts. The appearance was much the same at both two and seven days. In only one liver were scattered minute necrotic foci seen.

(*d*) *Biliary Obstruction Only.*—The five animals killed two days after obstruction had been created showed commencing bile duct proliferation and a slight increase of inflammatory cells in the portal tracts. Both liver parenchymal cells and bile duct epithelium were undergoing mitotic division. In only one section was there a small area of necrosis. After seven days, four of the five rats showed areas of focal hepatic necrosis, sometimes quite extensive (Figure II). Bile-duct proliferation was much more extensive than at two days (Figure III). Again, the portal tracts contained an increased number of inflammatory cells including polymorphs.

TABLE I

Group	Number of Rats	Procedure
I. Controls:		
(*a*)	5	None—normal rats
(*b*)	15	Sterile broth injection only
(*c*)	10	*B. coli* injection only
(*d*)	40	Biliary obstruction only
(*e*)	38	Biliary obstruction with sterile broth injection
II. Biliary obstruction and *B. coli* injection into portal vein:		
(*a*)	15	Injection at the time of the obstruction
(*b*)	9	Injection 7 days after creation of the obstruction
III. Biliary obstruction and *B. coli* injection into inferior vena cava:		
(*a*)	8	Injection at the time of obstruction
(*b*)	5	Injection 7 days after creation of the obstruction

In one animal of the five killed after nine days, several small parenchymal liver abscesses were present. At this time the necrotic areas in the liver had become a good deal more extensive and the bile ducts had proliferated even further. After 14 days, two of the five rats showed accumulations of pus cells in some of the smaller bile ducts, and necrosis of the parenchyma was prominent. By 21 days (three rats), bile-duct proliferation was even more prominent, the smaller branches insinuating themselves widely between the liver cells.

The 17 animals examined between 27 and 51 days showed similar changes (Figure IV). In addition, four of the rats showed collections of pus cells in some of the bile ducts (Figure V), two showed suppuration in necrotic areas, and in one, minute abscesses were seen to be centred around some small bile ducts.

(*e*) *Biliary Obstruction and Sterile Broth.*—In this group, broth was injected into the portal vein or the inferior vena cava either at the time of the obstruction or seven days later. Ten rats were given broth injections into the portal vein at the time of creating the obstruction; five of these were killed after two days and five after seven. In no case did the histo-

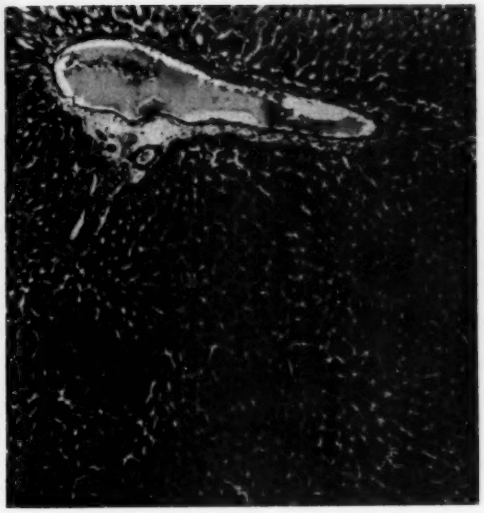


FIGURE I

Normal rat liver. The portal tract is surrounded by scanty fibrous stroma in which are a few lymphocytes. All sections are stained with hæmatoxylin and eosin. (×75)

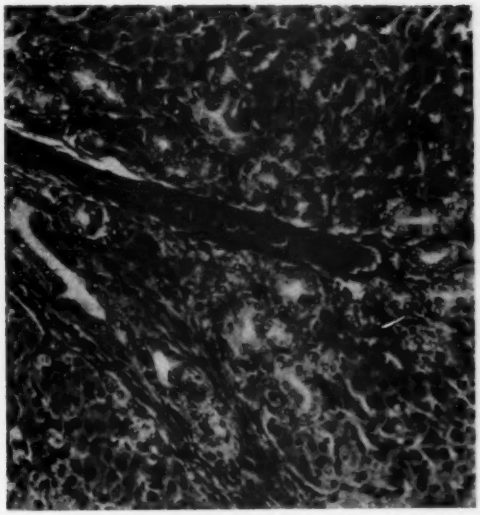


FIGURE III

From a control animal with biliary obstruction of seven days' duration. Bile duct proliferation forms a prominent feature. (×150)

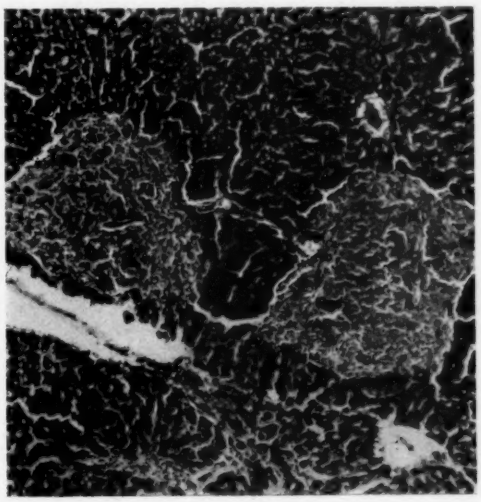


FIGURE II

From a control animal with biliary obstruction of seven days' duration. Two areas of focal necrosis are included. (×75)

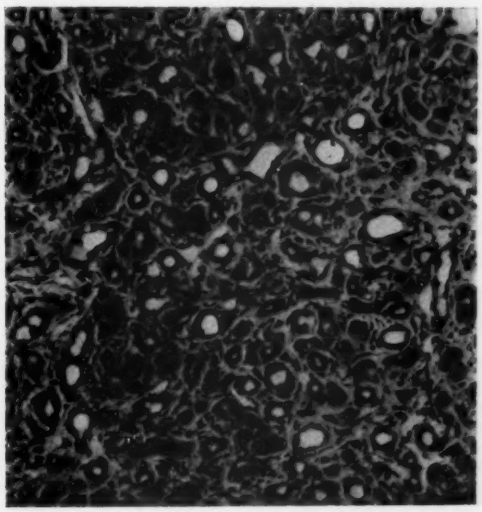


FIGURE IV

From a control animal with biliary obstruction of 44 days' standing. There are many dilated small bile ducts and canaliculi replacing a good deal of the liver parenchyma. (×150)

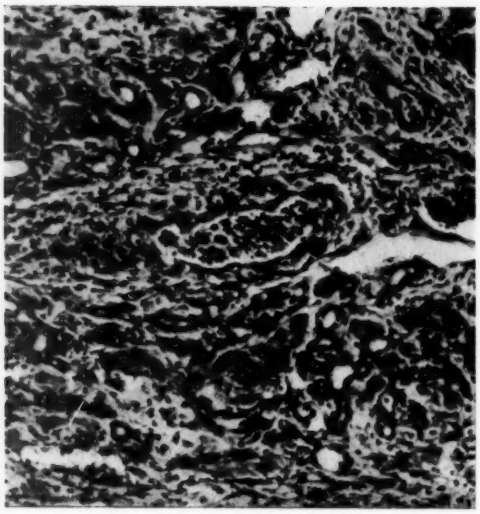


FIGURE V

From a control animal with biliary obstruction of 33 days' standing. The bile duct in the centre contains pus, and there is leucocytic infiltration in the surrounding connective tissue. (×150)

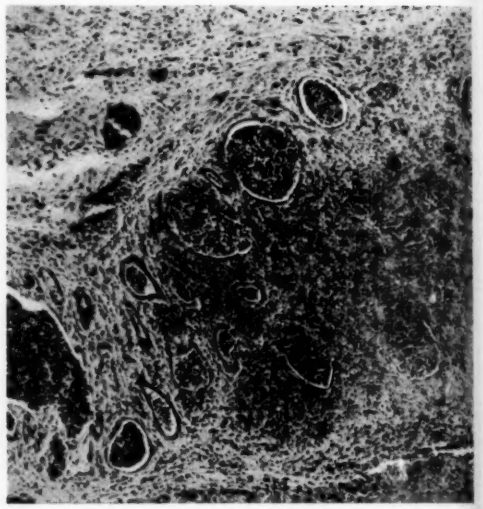


FIGURE VII

From the same liver as Figure VI. Suppurative cholangitis has spread to involve the substance of the liver. (×75)

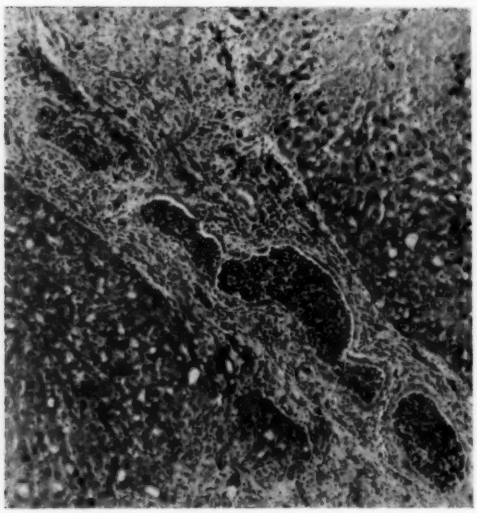


FIGURE VI

Biliary obstruction and immediate *B. coli* injection into the portal vein. This example, from an animal killed two days later, shows suppurative cholangitis. (×75)

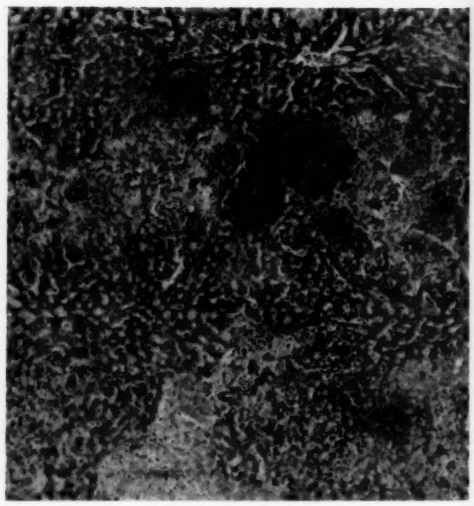


FIGURE VIII

Biliary obstruction followed one week later by *B. coli* injection into portal vein. This rat died within two days of the injection, and the liver contains many suppurative foci. (×75)

logical appearance in the liver differ from that seen with obstruction only. The same plan was adopted for sterile broth injection into the inferior vena cava with much the same results. Of the eight rats receiving broth injection into the portal vein a week after the establishment of the obstruction, two showed small collections of pus cells in the lumen of a few small bile ducts within seven days; otherwise the changes

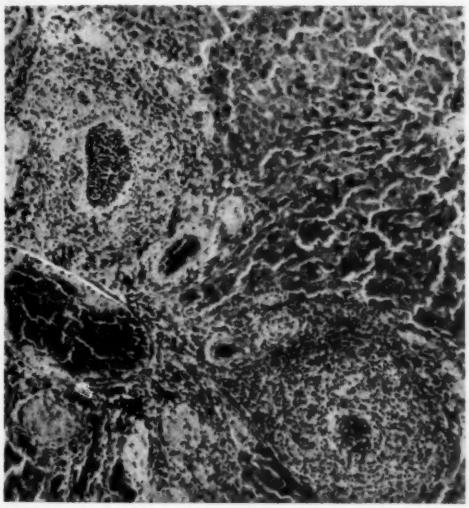


FIGURE IX

Biliary obstruction and immediate *B. coli* injection into the inferior vena cava. This specimen, from a rat killed seven days after the injection, shows widespread suppurative cholangitis. (×75)

resembled those following obstruction of equivalent duration without the broth. Finally, 10 rats received injections of sterile broth into the inferior vena cava seven days after obstruction. They were all examined two days later, and in only two cases were there a few small collections of polymorphs in the lumen of some of the smaller bile ducts. The usual changes resulting from obstruction alone were present in varying degrees.

Group II: Biliary Obstruction and B. Coli Injection into Portal Vein

(*a*) *Biliary Obstruction and Immediate B. Coli Injection.*—*B. coli* injection immediately after creation of the obstruction was performed in 15 rats. Ten of these were killed two days later, and in six suppuration in the liver was present (Figure VI). Of the remaining five surviving from three to nine days, all showed the macroscopic bile-stained abscesses of suppurative cholangitis (Figure VII).

(*b*) *Biliary Obstruction and Delayed B. Coli Injection.*—*B. coli* injection seven days after biliary obstruction was performed on nine rats. This proved to be a particularly lethal combination, for all animals died within two days of the injection. In spite of this, in five of the seven, abscesses in the liver were to be found (Figure VIII).

Group III: Biliary Obstruction and B. Coli Injection into the Inferior Vena Cava

(*a*) *Biliary Obstruction and Immediate B. Coli Injection.*—*B. coli* were injected immediately after creation of the obstruction, and of the five animals killed after two days, three showed liver abscesses. In another rat which died after three days, a liver abscess and obvious suppurative cholangitis were demonstrable. Of the remaining two killed after seven days, both showed suppuration in the liver (Figure IX).

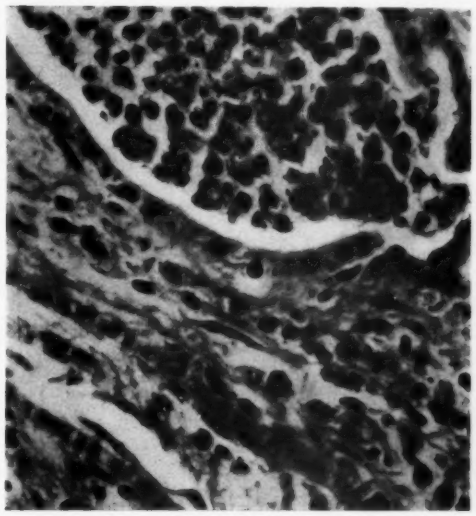


FIGURE X

Biliary obstruction followed by injection of *B. coli* into the inferior vena cava one week later. A bile duct is distended with pus. (×500)

(*b*) *Biliary Obstruction and Delayed B. Coli Injection.*—The final group of five rats received their *B. coli* injection into the inferior vena cava seven days after the obstruction. Unfortunately, all these animals died within two days; but in spite of this, two had already developed suppurative foci in the liver in the short interval between the injection and death (Figure X).

DISCUSSION

In this discussion, those specimens showing only an increase in periportal inflammatory cells were not classified as presenting evidence of infection. It was also found inadvisable to draw any distinction between suppurative cholangitis and other forms of liver abscess; indeed, it was sometimes impossible to achieve this clear differentiation. It was, therefore, thought reasonable in this experiment to consider these changes as one.

The effect of biliary obstruction alone was followed for periods of up to 51 days. Bile-duct proliferation had commenced by two days, and it progressed until, by about 30 days, the liver appeared to consist more of bile ducts than of parenchymal cells. Small areas of focal necrosis similar to those lesions described by Charcot and Gombault (1876) were a very common feature. They appeared after about two days and continued to do so for two to three weeks; but in some livers in which biliary obstruction was of long-standing they did not develop. Another form of necrosis described by Cameron and Oakley (1932) was often found. This was infarction of part or whole of an hepatic lobe in which thrombosis of the portal vein or some of its branches had occurred.

Of the 23 rats examined up to 21 days after obstruction, only three showed evidence of spontaneous inflammation. One of the livers after nine days showed a focus of suppuration in a small area of necrosis, and in two at 14 days, collections of pus cells were present in a few of the smaller branches of the bile ducts. Spontaneous infection was somewhat more frequent between 27 and 51 days, for of the 17 livers examined in this interval six contained foci of suppuration.

Injection of sterile broth into either the portal vein or the inferior vena cava at the time of the creation of the obstruction caused no changes differing from those obtained with obstruction alone. Of the 18 animals that received the broth injection one week after the obstruction, three were found to have scanty collections of pus cells in a few of the smaller bile ducts; this was approximately the same incidence as was found in animals with obstruction only of the equivalent duration.

When those rats are considered which had obstruction as well as receiving an intravenous injection of organisms, it can be seen that the incidence and extent of the inflammatory process were accentuated and their development greatly accelerated. In fact, of the 37 animals so treated, 23 (62%) had developed suppuration in the liver. As none of this particular group of rats survived for longer than nine days after obstruction, it is reasonable to compare these figures with those obtaining in controls with obstruction, with or without injection of sterile broth, examined at or before nine days. Of these 49 control animals, in only four (8%) were there any suppurative foci, and then they were only small. This difference in incidence of infection is highly significant ($P<0{\cdot}001$). Furthermore, when inflammation did occur, its intensity was greatly enhanced in the animals that received an injection of microorganisms after the creation of obstruction. Even after two days, injection of *B. coli* at the time of creating the biliary obstruction produced severe suppurative cholangitis, and quite large liver abscesses were often found. On the other hand, of those rats that had biliary obstruction but received no injection of microorganisms, the first abscess to appear was at nine days, and in the others showing evidence of infection it consisted only of a collection of a few polymorphs in an ocasional small bile duct.

It can be seen from the foregoing results that biliary obstruction predisposes to infection. The evidence also indicates that infection following biliary obstruction is of hæmatogenous origin, for the so-called "ascending" route has been eliminated as a possibility by the operative procedure followed for the creation of the biliary obstruction. It is true that spontaneous infection (albeit rather mild) did occur occasionally in rats with obstruction alone, just as is seen in the human disease; this probably resulted from naturally occurring transient bacteriæmia.

ACKNOWLEDGEMENT

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REFERENCES

CAMERON, G. R., and OAKLEY, C. L. (1932), "Ligation of the Common Bile Duct", *J. Path. Bact.*, **35**, 769.

CHARCOT, J. M., and GOMBAULT, A. (1876), *Arch. physiol. norm. path.*, **3**, 272; quoted by Cameron, G. R., and Oakley, C. L., *loco citato*.

HIMSWORTH, H. P. (1954), "Lectures on the Liver and its Diseases", 2nd Edition, Blackwell, Oxford.

NATUCCI, G. (1935), "*Se la stasi bilare predisponga il fegato alla infezione ematogena*", *Sperimentale*, **89**, 272.

TRAMS, E. G., and SYMEONIDIS, A. (1957), "Morphologic and Functional Changes in the Livers of Rats after Ligation or Excision of the Common Bile Duct", *Amer. J. Path.*, **33**, 13.

UMBER, F., and HEINE, K. (1924), "*Experimentelle Studien zur Cholangiefrage*", *Arch. exp. Path. Pharmak.*, **103**, 329.

VON KALK, H., and WILDHIRT, E. (1956), "*Cholangitis-Choledochusstein*", *Dtsch. med. Wschr.*, **81**, 1253.

WILKIE, A. L. (1928), "The Bacteriology of Cholecystitis; a Clinical and Experimental Study" *Brit. J. Surg.*, **15**, 450.

PLASMA TRANSAMINASE IN LIVER DISEASE[1]

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Summary

The plasma glutamic oxalacetic transaminase level (G-O.T.) was estimated in 206 cases of liver disease. Levels over 500 units favoured a diagnosis of acute viral hepatitis; but levels of this order and higher occurred in active chronic hepatitis and lupoid hepatitis during an exacerbation. Levels between normal and 200 units were found in all forms of liver disease.

Serial G-O.T. estimations proved of considerably greater diagnostic and prognostic aid than single readings, and were of value in studying the progress of liver disease, the assessment of therapeutic response and the detection of sensitivity to hepatotoxic drugs.

The value of G-O.T. estimation in obstructive jaundice was limited, since the results overlapped those found in parenchymal liver disease

The histological finding of hepatocellular necrosis was never associated with normal G-O.T. levels; but no close association could be shown between the degree of liver cell necrosis and the G-O.T. level.

Wroblewski and La Due (1954) reported elevated levels of glutamic oxalacetic transaminase (G-O.T.) in the serum of patients with liver disease; absolute levels and serial alterations in G-O.T. activity proved to be of value in the diagnosis of liver disease (Wroblewski and La Due, 1955; Chinsky *et alii*, 1956). Molander *et alii* (1955) and Friend *et alii* (1956) showed that the level of G-O.T. activity correlated broadly with liver cell necrosis produced experimentally.

This paper presents the results of plasma glutamic oxalacetic transaminase (P.G-O.T.) estimations in 206 cases of liver disease of various types. We have assessed the diagnostic value of absolute levels and serial changes in P.G-O.T. activity in liver disease, and have compared these findings with histological changes in the liver.

Methods

Most patients were in hospital receiving a routine diet. A few were out-patients. Twelve patients received treatment with cortisone. Blood was taken for P.G-O.T. estimation one day after admission to hospital and daily thereafter. P.G-O.T. assay was performed by a modification (O'Brien and Goble, 1958) of the method of Karmen (1955). Values were corrected for temperature to 20° C. The range of values in 200 normal subjects found by this method was 5 to 28 units per ml. with a mean of 11 (S.D. ± 4). The maximum day-to-day variation in normal subjects was 15 units (Goble and O'Brien, 1958).

Liver tissue was available for histological comparison with P.G-O.T. levels in 48 cases. Tissue was obtained by needle or operation biopsy, which was done for indications unrelated to this study, and at autopsy. The salient pathological features—namely, necrosis of liver cells, regeneration of liver cells and cellular infiltration—were recorded semi-quantitatively as —, +, ++, +++, ++++. Under the heading of "necrosis", cellular damage short of actual death was taken into account. A glycogen stain was included, since we consider loss of glycogen to be an indication of cellular damage. Regenerative activity was assessed by increased numbers of binucleate cells and giant cells of bizarre appearance, the latter often showing hyperchromatic and multiple nuclei and cytoplasmic basophilia.

The time relation between biopsy and P.G-.O.T. estimation was recorded because a high level of enzyme is more transient than the cell damage causing enzyme release; that is, histological evidence of necrosis may be present after the P.G-O.T. level has returned to normal.

Results

The results of serial P.G-O.T. estimations in 206 cases grouped according to the final diagnosis are presented in Table I. The table includes the number of cases in each group, the highest and lowest individual values for P.G-O.T., and the ranges and means for each group. The lowest value recorded for each

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[6] Working with the aid of a grant from the Life Insurance Medical Research Fund of Australia and New Zealand.
[7] Working with the aid of a grant from the National Health and Medical Research Council of Australia.

TABLE I

Results of Serial P.G-O.T. Determinations in Cases of Liver Disease

Disease	Number of Cases	Peak Values (Units)		Lowest Values (Units)	
		Range	Mean	Range	Mean
Acute viral hepatitis	32	1472–38[1]	364	168–39	81
Cholangiolitic hepatitis	6	344–56	147	114–33	64
Active chronic hepatitis	26	4400–16	86[2]	122– 9	38
Lupoid hepatitis	10	572–65	263	41–13	25
Nutritional hepatitis	31	164–10	60	40– 9	25
Obstructive jaundice	27	166–13[1]	74	55–20	37
Biliary cirrhosis	9	330–25	101	48–25	35
Cholangitis	11	151–25	65	—	—
Hepatic carcinoma	10	166–30	78	55–21	35
Chlorpromazine jaundice	8	232–29	106	—	—
Hepatic tuberculosis	1	20	—	—	—
Amœbic hepatitis	1	8	—	—	—
Hæmochromatosis	6	260–32	77	130–15	49
Hepatic hydatid disease	1	40	—	13	—
Septicæmia	1	56	—	30	—
Pyrazinamide toxicity	6	135–32	57	—	—
Dubin-Johnson syndrome	1	12	—	—	—
Glandular fever	1	140	—	—	—
"Q" fever	4	66	—	38	—
Pernicious anæmia (with jaundice)	1	52	—	10	—
Chronic hæmolytic anæmia (with jaundice)	1	18	—	—	—
Cardiac failure with hepatomegaly	6	58–11	28	—	—
Acute myeloid leukæmia	1	10	—	—	—
Chronic myeloid leukæmia	2	35–16	—	—	—
Chronic lymphatic leukæmia	2	16–10	—	—	—
Wilson's disease	1	39–17	—	—	—

[1] The low values indicated are single estimations and are therefore treated as peak values.
[2] The value 4400 was not used in calculating this mean. It was considered that the result would give an erroneous impression. The highest value obtained in other cases was 572.

individual was the lowest value obtained whilst the disease remained active. If only one estimation was performed, this was taken as the highest value. The highest values of 125 patients in the most common groups are illustrated in Figure I.

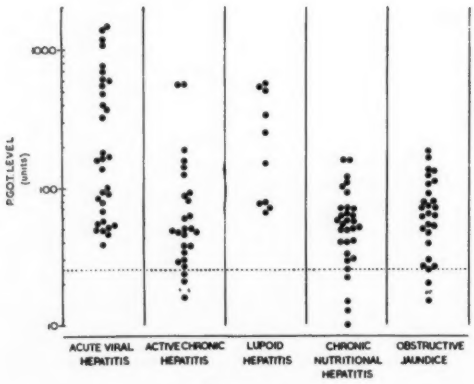


FIGURE I

The highest P.G-O.T. levels (log scale) of 125 patients in the most common forms of liver disease studied

The levels of P.G-O.T. were highest in the 32 cases of acute viral hepatitis. High initial values fell sharply over the succeeding seven to 21 days to the normal range (Figure II).

The levels of P.G-O.T. in active chronic hepatitis (Saint *et alii*, 1953) varied considerably from high values during exacerbations to normal values during remissions. Most cases showed mild to moderate elevations (50 to 200

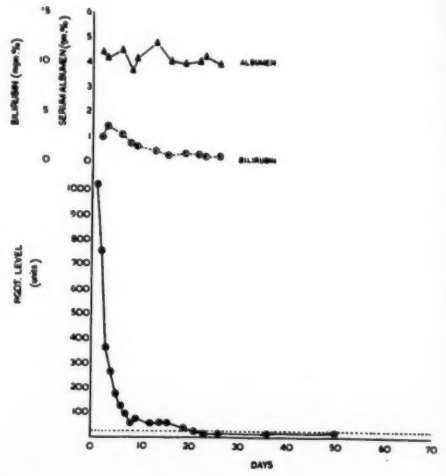


FIGURE II

Serial P.G-O.T. levels in a typical case of acute viral hepatitis in a man, aged 19 years. There was a steep fall during the first week, with a progressively slower return to normal over the next two weeks

units) (Figure III) during the period of observation. One patient with active chronic hepatitis had the highest value obtained in the entire series (4400 units); this fell gradually to 74

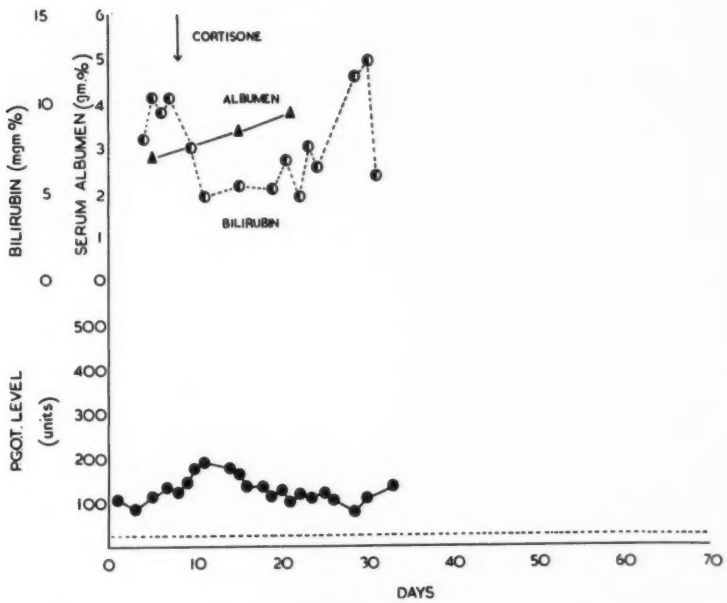


FIGURE III

Serial P.G-O.T. levels in a case of active chronic hepatitis in a man, aged 32 years, showing a level persistently elevated above normal, unaltered by cortisone treatment

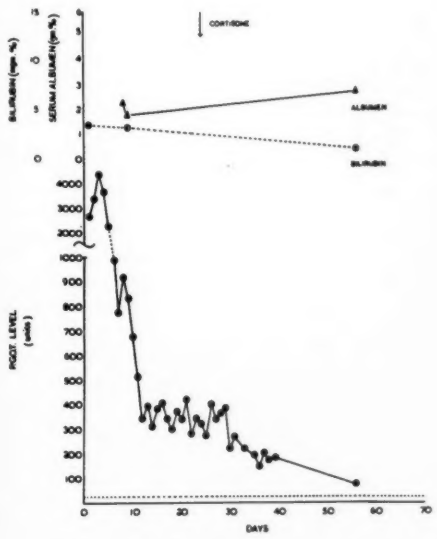


FIGURE IV

Serial P.G-O.T. levels in a girl, aged 17 years, with active chronic hepatitis, in whom the highest P.G-O.T. in the series was recorded. The gradual fall in level corresponded with clinical improvement. Response to cortisone was uncertain

units over the succeeding two months, coincidentally with remission (Figure IV).

There were 10 cases of lupoid hepatitis. This category comprised cases of active chronic hepatitis in which a positive response to the L.E. cell test was obtained on at least one occasion; some also showed certain features of systemic lupus erythematosus (Mackay *et alii*, 1959). P.G-O.T. values in lupoid hepatitis tended to be higher than in active chronic hepatitis (Figure V).

Values in chronic nutritional hepatitis (Wood *et alii*, 1949) during exacerbations were usually lower than those obtained in active chronic and lupoid hepatitis (Figure VI).

In obstructive jaundice, P.G-O.T. levels were generally lower than those obtained in acute and chronic hepatitis, but there was considerable overlap. Raised levels of P.G-O.T., therefore, proved only of limited value in distinguishing biliary obstruction from parenchymal liver disease. This was particularly so in biliary obstruction associated with biliary cirrhosis, cholangitis and hepatic carcinoma. High values (greater than 200 units) were obtained in three of the seven cases of chlorpromazine jaundice, indicating that liver-cell damage is associated with cholestasis in this condition, as suggested by Zelman (1959).

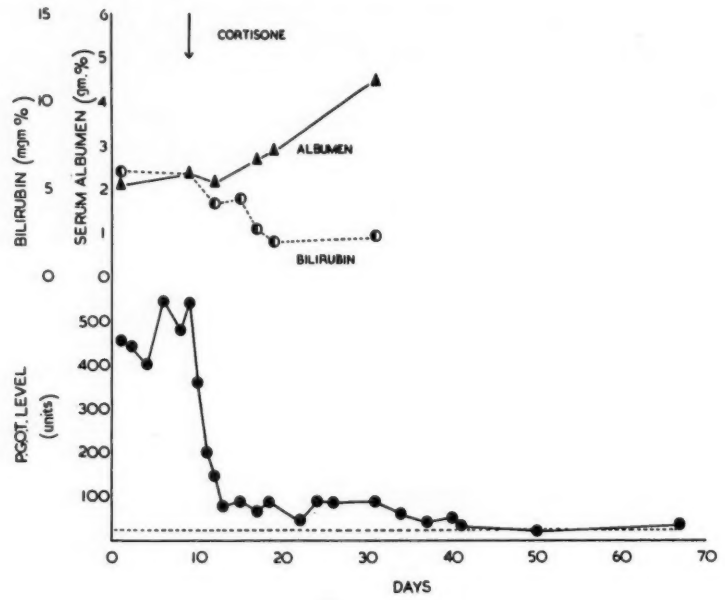


FIGURE V

Serial P.G-O.T. levels in a woman, aged 28 years, with lupoid hepatitis, during an exacerbation. The P.G-O.T. level fell sharply when cortisone was given

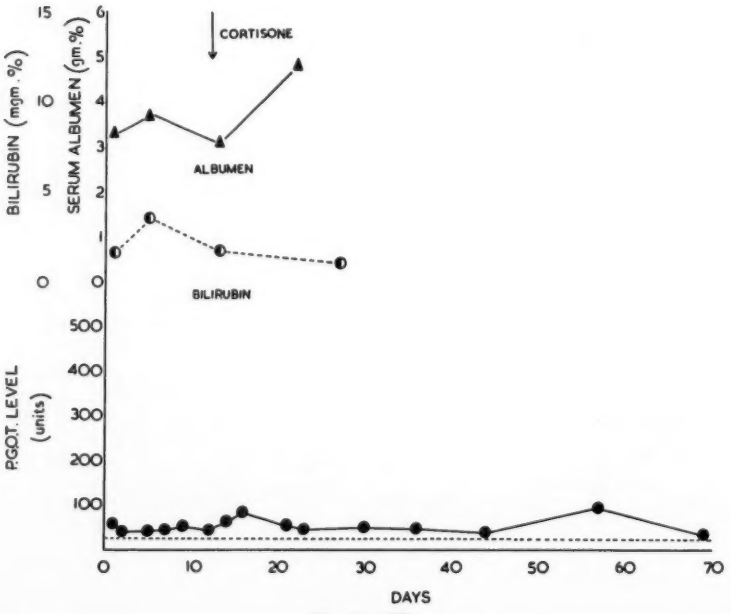


FIGURE VI

Serial P.G-O.T. levels in an alcoholic woman, aged 36 years, with chronic nutritional hepatitis admitted in coma. The elevation of P.G-O.T. level was mild (compare Figure III) and uninfluenced by treatment with cortisone

The single high P.G-O.T. level of 260 units in six cases of hæmochromatosis occurred in a case in which there was an associated hepatoma.

Elevation of the P.G-O.T. level of one patient with hydatid cysts in the liver occurred on the day after surgical removal of a cyst. Four other estimations in this case gave figures within normal limits.

Six of 24 patients receiving pyrazinamide as an antituberculosis drug showed elevated levels in serial estimations. In five the rise was slight and did not persist after cessation of the drug. In one the level remained moderately raised for six weeks after cessation of the drug, and the patient ultimately died in liver failure (Price, 1959).

Slight to moderate elevation of P.G-O.T. levels were observed in "Q" fever, cardiac failure with hepatomegaly and other miscellaneous conditions (see Table I).

Five patients were investigated during liver coma due to chronic hepatitis (3), hæmochromatosis (1) and pyrazinamide toxicity (1); the depth of coma bore no relation to the P.G-O.T. level.

Administration of cortisone produced a significant fall in P.G-O.T. levels in four cases of active chronic hepatitis—three were of the

TABLE II

Relation of P.G-O.T. Levels to Histological Findings in the Liver

Disease	PG-O.T. Level (Units)	Liver Biopsy[1]				
		N.	R.	C.I.	F.	Time Relation to P.G-O.T.[2]
Acute viral hepatitis ..	62	+	−	++	−	+1
	50	++	+++	+++	+	−3
	1016	++	++	+++	−	+12
Active chronic hepatitis ..	50	+	−	+	−	0
	37	++	+++	+++	+	0
	136	+++	+	+++	+	−1
	43	++	+	++	−	−8
	23	−	−	++	−	+3
	114	+++	++	+++	−	−4
	48	+	+	++	−	−3
	44	+	+	+	−	+8
	92	++	++	++	−	+7
Lupoid hepatitis	302	++	+++	+++	−	0
	86	++	+++	+++	−	0
	93	++	++	+++	−	0
	30	+	−	++	−	+5
	35	+	−	+++	−	+7
	540	+++	+	+++	−	0
	44	+	−	++	−	0
Nutritional hepatitis ..	109	+	−	+++	++	+24
	48	+	−	++	+++	0
	70	+	−	++	+	+4
	10	−	−	−	++	+6
	26	−	−	+	+	+6
	33	+	−	++	+	+2
	13	−	−	−	+	+12
	65	+	++	+++	+++	+7
	41	+	−	+++	+++	+4
	55	+	++	+++	+++	−11
Obstructive jaundice.. ..	122[3]	++	+	++	−	0
	85	+	+	++	−	0
	54[4]	+	−	+	−	+2
	72[5]	+	−	++	−	+9
	27[6]	−	−	++	−	+5
	26	−	−	+	−	+3
Biliary cirrhosis	70	+	+	++	−	+4
	45[7]	+	+	++	−	+3
Chlorpromazine jaundice ..	25	++	+	++	−	−9
	164	+	−	+	++	+1
	37	+	−	+	−	0
Hæmochromatosis	260	++	−	++	−	+14
	42	+	−	++	−	+1
	50	+	−	++	−	−6
	32	+	−	++	−	+13
	25	−	−	++	−	+2
Dubin-Johnson syndrome ..	12	−	−	−	−	+5
Q" fever	66	+	−	+	−	+2
	47	+	−	+	+	0
	38	+	−	+	+	+1
	38	+	−	++	+	−4

[1] N., necrosis; R., regeneration; C.I., cellular infiltration; F., fatty change; −, none; +, slight; ++, moderate; +++, considerable; ++++, gross.
[2] Time relation to P.G-O.T.: +1, 1 day later; −1, 1 day earlier; 0, same day.
[3] Carcinoma of the hepatic duct, portal vein thrombosis, infarction (Taft, 1959).
[4] Pancreatic carcinoma, liver metastases.
[5] Carcinoma of the colon, liver metastases.
[6] Cholelithiasis.
[7] Carcinoma of the common bile duct.

lupoid type (Figure V). No such fall occurred in four other cases of active chronic hepatitis (one of the lupoid type), or in two cases of nutritional hepatitis, one case of obstructive jaundice and one case of hæmochromatosis.

Table II compares the histological appearances of the liver and the P.G-O.T. level closest to the date of biopsy. Necrosis of gross degree—that is, ++++— did not occur in this series.

The comparison between P.G-O.T. level and the degree of necrosis and regeneration of the liver is shown in Figure VII. Patients without histological evidence of necrosis with one exception had a normal P.G-O.T. level. Patients

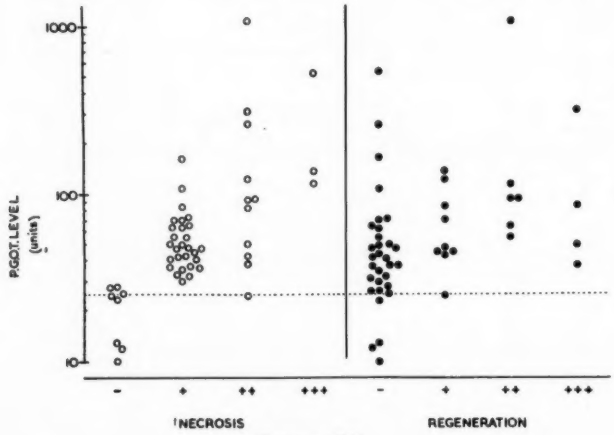


FIGURE VII

Comparison of P.G-O.T. levels (log scale) with the degree of liver cell necrosis and regeneration in 48 cases of liver disease. There was correlation between P.G-O.T. levels and the presence or absence of necrosis, but no close correlation with the degree of necrosis. There was no correlation between P.G-O.T. levels and regeneration

showing necrosis invariably had a raised P.G-O.T. level. However, P.G-O.T. levels did not correlate closely with the degree of necrosis. There was a slight but unimpressive correlation with cellular infiltration, but none with liver-cell regeneration or fatty change.

DISCUSSION

It was our experience that serial G-O.T. estimations were more valuable than single readings, although single G-O.T. levels greater than 500 units usually indicated acute viral hepatitis (Wroblewski and La Due, 1956; de Ritis *et alii*, 1955; Chinsky *et alii*, 1956; Madsen *et alii*, 1958; Price-Davies and Wilkinson, 1958). However, values over 500 units occurred in some of our cases of active chronic and lupoid hepatitis. Isolated levels between 200 and 500 units favoured parenchymal liver disease rather than uncomplicated extrahepatic biliary obstruction. Levels between 25 and 200 units were found in all forms of liver disease, including biliary obstruction, and thus *per se* did not differentiate parenchymal disease from biliary obstruction. However, normal levels in the presence of jaundice were always associated with extrahepatic biliary obstruction.

Serial G-O.T. estimations were useful in distinguishing acute viral hepatitis from other forms of parenchymal liver disease, in that a high level followed by a steady fall (within 21 days) to normal levels was suggestive of acute hepatitis, whereas a slow and intermittent fall occurred with other forms of liver disease. Serial estimations obviated the necessity of estimating the peak level.

The greatest value of serial G-O.T. estimations appeared to be in assessing the progress of liver disease. As the enzyme disappears from the circulation rapidly in the absence of cellular injury (see Goble and O'Brien, 1958), it is a much more sensitive index of continuing liver damage than conventional tests, such as cephalin flocculation, zinc sulphate turbidity and thymol turbidity, the results of which frequently remain positive long after cellular injury has ceased. Serial estimations were also of value in assessing the response to therapy (O'Brien *et alii*, 1958) and in demonstrating the toxic effects of drugs, as was shown here with

pyrazinamide. Delkeskamp *et alii* (1959) demonstrated the value of G-O.T. estimations in the detection of non-icteric and incipient acute viral hepatitis. We had two such cases in this series. The progress of acute viral hepatitis to the chronic phase should be readily detectable by this method. Some indication of prognosis was obtained from serial estimations. Prolonged elevations in P.G-O.T. in any form of liver disease indicated an unfavourable prognosis.

Our results were not in accord with the findings of Molander *et alii* (1955), Friend *et alii* (1956), and Zelman and Wang (1959), which suggested that G-O.T. levels were proportional to the degree of liver-cell necrosis. In some instances this discrepancy was undoubtedly due to the time interval between biopsy and estimation. In our opinion, however, it is virtually impossible to correlate G-O.T. release, which is known to be transient in the absence of continuing cellular injury, with a histological change which may take days to develop and weeks to disappear. Moreover, liver tissue obtained at biopsy may not have been a representative sample. In those cases in which the P.G-O.T. level was much higher than was expected from the degree of necrosis, it is possible that G-O.T. was produced in increased quantity by surviving liver cells and subsequently leaked into the circulation through damaged cellular membranes. This concept has been advanced by Gerlach (cited by Leppelmann, 1958), when he showed that in acute viral hepatitis, serum levels of sorbit dehydrogenase exceeded the quantity of this enzyme known to be present in normal liver.

Although we were unable to demonstrate close correlation between P.G-O.T. levels and the degree of liver-cell necrosis, it is highly significant that patients with histological evidence of necrosis showed abnormal P.G-O.T. values whilst patients with normal histological findings showed normal values. Similar results were obtained by Zinke and Leevy (1958) and by Arturson and Persson (1959). We suggest that P.G-O.T. level does not necessarily differentiate between cellular injury and cellular destruction or breakdown.

No correlation was found between liver-cell regeneration and P.G-O.T. levels. We expected this on clinical grounds, because in the recovery phase of acute viral hepatitis, when regeneration is most active, P.G-O.T. levels were falling to normal. Thus, although Greenbaum *et alii* (1954) have shown that regenerating cells contain an increased quantity of G-O.T., this is not necessarily liberated into the plasma.

ACKNOWLEDGEMENTS

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REFERENCES

ARTURSON, G., and PERSSON, B. H. (1959), "Serum Transaminase Activity as an Index of Liver Cell Destruction", *Acta chir. scand.*, **117**, 221.

CHINSKY, M., SHMAGRANOFF, G. L., and SHERRY, S. (1956), "Serum Transaminase Activity: Observations in a Large Group of Patients", *J. Lab. clin. Med.*, **47**, 108.

DELKESKAMP, A., SCHMIDT, E., and SCHMIDT, F. W. (1959), "*Fermentaktivitäts—Bestimmungen in der Diagnostik der anikterischen Hepatitis*", *Dtsch. Med. Wschr.*, **84**, 188.

DE RITIS, F., COLTORTI, M., and GUISTI, G. (1955), "*Altivita transaminasica del siero umano nell'epatite virale*", *Minerva Med. (Torino)*, **1**, 1207.

FRIEND, C., WROBLEWSKI, F., and LA DUE, J. S. (1955), "Glutamic Oxalacetic Transaminase Activity of Serum in Mice with Viral Hepatitis", *J. exp. Med.*, **102**, 699.

GOBLE, A. J., and O'BRIEN, E. N. (1958), "Acute Myocardial Ischæmia: Significance of Plasma Transaminase Activity", *Lancet*, **2**, 873.

GREENBAUM, A. L., GREENWOOD, F. C., and HARKNESS, R. D. (1954), "Glutamic Dehydrogenase and Glutamic Aspartic Transaminase in Regenerating Liver of the Rat", *J. Physiol.*, **125**, 251.

KARMEN, A. (1955), "A Note on the Spectrophotometric Assay of Glutamic Oxalacetic Transaminase in Human Blood Serum", *J. clin. Invest.*, **34**, 131.

LEPPELMANN, H. J. (1958), Discussion of the paper by Henley, K. S., Wiggins, H. S., Pollard, H. M., and Dullaert, E., *Ann. N.Y. Acad. Sci.*, **75**, 273.

MACKAY, I. R., TAFT, L. I., and COWLING, D. C. (1959), "Lupoid Hepatitis and the Hepatic Lesions of Systemic Lupus Erythematosus", *Lancet*, **1**, 65.

MADSEN, S., BANG, N. U., and IVERSON, K. (1958), "Serum Glutamic Oxalacetic Transaminase in Diseases of the Liver and Biliary Tract", *Brit. med. J.*, **1**, 543.

MOLANDER, D. W., WROBLEWSKI, F., and LA DUE, J. S. (1955), "Serum Glutamic Oxalacetic Transaminase as an Index of Hepatocellular Integrity", *J. Lab. clin. Med.*, **46**, 831.

O'BRIEN, E. N., and GOBLE, A. J. (1958), unpublished data.

O'BRIEN, E. N., GOBLE, A. J., and MACKAY, I. R. (1958), "Plasma Transaminase Activity as an Index of the Effectiveness of Cortisone in Chronic Hepatitis", *Lancet*, **1**, 1245.

PRICE, C. G. (1959), unpublished data.

PRICE-DAVIES, J., and WILKINSON, J. H. (1958), "Diagnostic Value of Serum Transaminase Activity in Hepatic and Gastro-intestinal Diseases", *Lancet*, **1**, 1249.

SAINT, E. G., KING, W. E., JOSKE, R. A., and FINCKH, E. S. (1953), "The Course of Infectious Hepatitis with Special Reference to Prognosis and the Chronic Stage", AUST. ANN. MED., **2**, 113.

TAFT, L. I. (1959), "Left Lobar Biliary Cirrhosis and Right Lobar Ischæmic Necrosis of the Liver due to Cholangiocarcinoma", *J. Path. Bact.*, **78**, 279.

WOOD, I. J., GARLICK, H. W., MOTTERAM, R., WEIDEN, S., MOORE, A., MACKAY, M., and TURNER, C. N. (1949), "Nutritional Disease of the Liver", *Med. J. Aust.*, **1**, 541.

WROBLEWSKI, F., and LA DUE, J. S. (1954), "Serum Glutamic Oxalacetic Transaminase as an Index of Liver Cell Injury", *J. Lab. clin. Med.*, **44**, 958.

WROBLEWSKI, F., and LA DUE, J. S. (1955), "Serum Glutamic Oxalacetic Transaminase Activity as an Index of Liver Cell Injury: A Preliminary Report", *Ann. intern. Med.*, **43**, 345.

WROBLEWSKI, F., and LA DUE, J. S. (1956), "Serum Glutamic Oxalacetic Aminopherase (Transaminase) in Hepatitis", *J. Amer. med. Ass.*, **160**, 1130.

ZELMAN, S. (1959), "Liver Cell Necrosis in Chlorpromazine Jaundice (Allergic Cholangiolitis)—A Serial Study of 26 Needle Biopsy Specimens in Nine Patients", *Amer. J. Med.*, **27**, 708.

ZELMAN, S., and WANG, C. C. (1959), "Transaminase in Serum and Liver Correlated with Liver Cell Necrosis in Needle Aspiration Biopsies", *Amer. J. med. Sci.*, **237**, 323.

ZINKE, M. R., and LEEVY, C. M. (1958), "Correlation of Serum Transaminase with Histopathology in Laennec's Cirrhosis", *Gastroenterology*, **34**, 1046.

A CASE OF PROTEIN-LOSING ENTEROPATHY[1]

ZELMAN FREEMAN[2]

SUMMARY

The case described is of a woman, aged 61 years, presenting with diarrhœa, fever and œdema associated with hypoproteinæmia. Biopsy of the intestine showed acute non-specific ileitis, and biopsy of the liver showed severe fatty degeneration. Laparotomy revealed some vascular engorgement of the subserosal vessels in the lower ileal area. After the commencement of prednisone therapy, there was considerable improvement in the clinical features, the hypoproteinæmia and the histological appearances in the liver. In the state of remission, studies with radioactive labelled albumin showed a degradation rate of 0·46 gramme per day per kilogram, and studies with radio-iodinated polyvinylpyrrolidone showed a fæcal loss of 2·9% in 96 hours. Both figures are twice to three times normal.

HYPOPROTEINÆMIA due to a lowering of the plasma albumin level is a common finding in many conditions, such as the nephrotic syndrome, advanced liver disease, malnutrition, malabsorption and ulcerative colitis. Occasionally no such obvious cause is present, and such cases in the past have been referred to as examples of idiopathic hypoproteinæmia. Protein loss from the plasma into the intestine, or "protein-losing enteropathy", is a newly-described syndrome associated with a lowering of the serum protein level. Gastro-intestinal symptoms may or may not be present, and œdema is a frequent accompaniment. The discovery of the mechanism of this condition took place in several steps. The first was the finding by Albright *et alii* (1950) that hypoproteinæmia could be due to the rapid disappearance of proteins from the plasma rather than to impaired synthesis in the liver. Schwartz and Thomsen (1957) described a case in which they injected isotopically labelled albumin intravenously, and noticed a more rapid disappearance of the labelled albumin from the plasma than in normal controls, and in the absence of any obvious external loss they ascribed the disappearance of the albumin to hypercatabolism. More recently the problem of idiopathic hypoproteinæmia has been reviewed by Gordon *et alii* (1959), who described the two following types: (i) an anabolic type due to impaired synthesis of albumin; (ii) a hypercatabolic type due to an increased rate of degradation of the albumin, either by deamination of the amino acids or by their conversion to a phosphorus-containing tissue in the form of protoplasm. Steinfield *et alii* (1957) showed that loss of serum proteins into the bowel occurred with regional enteritis and ulcerative colitis. Holman *et alii* (1959) also showed by radioactive studies and immunological techniques that intestinal fluid recovered from the jejunum in six cases of idiopathic hypoproteinæmia contained serum protein, but they also found it to be present in normal subjects. However, the loss was excessive in the former group. The next stage came with the development by Gordon (1959) of a diagnostic tool which could indicate a leak of protein into the alimentary canal. This was radio-iodinated polyvinylpyrrolidone (I^{131} P.V.P.) with a molecular weight similar to that of albumin. When it is injected intravenously, if a "leak" is present this substance finds its way into the alimentary canal, where it resists digestion and retains the I^{131}, unlike I^{131}-labelled albumin, which is digested and resorbed. The radioactivity of the fæces can then be measured (*vide infra*). Normally it lies between 0·8% and 1·6% of the administered dose. In the case of protein-losing enteropathy, the severity of the hypoproteinæmia and the degree of recoverability of the labelled P.V.P. are proportional, and in such patients the latter ranges from 2·9% to 20% of the administered dose. Whilst the albumin fraction is the one mainly lost from the plasma, studies with isotopically-labelled globulin show that a slight leak of this fraction also occurs; but the technique is not adequate for detailed studies (Gordon *et alii*, 1959).

The following case, investigated with both I^{131}-labelled P.V.P. and I^{131}-labelled albumin, is, as far as I am aware, the first reported demonstration in Australia by these techniques of serum albumin loss into the gut.

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CASE REPORT

On July 8, 1958, a woman, aged 61 years, consulted her doctor complaining of pains in her joints. She was given 50 mg. of " Myocrisin ", and a similar dose was given again on July 28. In the following two weeks she developed a non-specific rash and diarrhœa, which led to her admission to the Prince Henry Hospital, Sydney, on August 18. In addition to diarrhœa, she had vomiting and colicky abdominal pain. This settled down to severe intermittent diarrhœa, with the frequent passage of loose brown watery stools containing no blood or mucus. Her previous illnesses included thyroidectomy in 1953 because of a " nodule ", and appendicectomy at the age of 21 years. Her family history was not relevant. Physical examination gave essentially negative results, and no œdema was present. A number of tests were performed on this occasion with the following results. The blood urea content was 30 mg. per 100 ml. A random blood sugar estimation was 90 mg. per 100 ml. Tests for occult blood gave negative results. The hæmoglobin value was 12·4 grammes per 100 ml. The leucocytes numbered 8000 per cubic millimetre, and were of normal distribution. There was slight anisocytosis in the erythrocytes. The stools contained no ova; pathogens were not detected. A further stool culture yielded a *Salmonella*, of a type not determined. No tubercle bacilli were detected in the sputum on smear or cultural examination. X-ray examination with a barium enema gave normal results apart from some irritability of the colon. The X-ray film of the chest was clear. The patient had achlorhydria, which was histamine-fast. She left hospital of her own accord.

About a month later she was admitted to the Eastern Suburbs Hospital complaining of epigastric pain and diarrhœa, which had continued. She was febrile at this time, with her temperature rising to 102° at night. The fever gradually settled down over the next four weeks. During this time she passed four to six stools a day. Four weeks after her admission to hospital, ankle œdema appeared, which spread up to her thighs. She appeared rather plump, with a somewhat bloated facies, and throughout the illness there was a purplish-red colour on her cheeks. Apart from some epigastric tenderness, clinical examination gave negative results. A sigmoidoscopic examination showed granular, slightly reddened mucosa with a grey mucoid offensive discharge, and on a later occasion no abnormality was apparent. A second chest X-ray examination revealed no abnormality; the same result was obtained at two barium enema X-ray examinations, one using an air contrast medium. The result of a Graham's test also was negative. Five stools were examined for cysts and pathogens, and all results were negative. The test for occult blood gave a negative result. The hæmoglobin value was 12·2 grammes per 100 ml. The erythrocytes numbered 4,100,000 per cubic millimetre, and the erythrocyte sedimentation rate was 9 mm. in one hour. The leucocytes numbered 6300 per cubic millimetre, and were of normal distribution. The serum alkaline phosphatase content was 9·2 King-Armstrong units, the thymol turbidity was 0 to 1 unit, and the zinc sulphate turbidity was 1 to 2 units. " Bromsulphalein " retention was 47% at 30 minutes. The serum bilirubin content was 0·6 mg. per 100 ml. Agglutination tests against typhoid, paratyphoid and brucellosis were carried out, but gave negative results. The serum electrolytes were normal, and a blood culture yielded a growth of *Bacterium coli* after 10 days. Microscopic examination of the urine showed 10 to 12 pus cells per high-power field, an occasional red cell and fairly numerous hyaline casts. Culture yielded *Proteus vulgaris*. The total serum protein content was estimated at 3·7 grammes per 100 ml. (albumin 1·59, alpha-1 globulin 0·14, alpha-2 globulin 0·40, beta globulin 0·74, gamma globulin 0·88). One month after her admission to hospital the patient's hæmoglobin value dropped to 9·6 grammes per 100 ml., with a normal leucocyte count. During this time the following therapy was given: streptomycin, phthalylsulphathiazole, chloramphenicol, erythromycin, penicillin, emetine, vitamins and " Kaomagma ", all without any significant effect on either the diarrhœa or the fever.

On November 18, three months after the onset of symptoms, she was admitted to the Special Unit of the Prince of Wales Hospital for further investigation, with a diagnosis of possible lymphoma of the intestine. On examination of the patient, her weight was 110 lb. and her height 59 in. There was no loss of body hair, and there was no physical abnormality to be detected. Her total serum protein content was 4·4 grammes per 100 ml., and the albumin fraction was low (Figure I). Her temperature was 101° F., but this

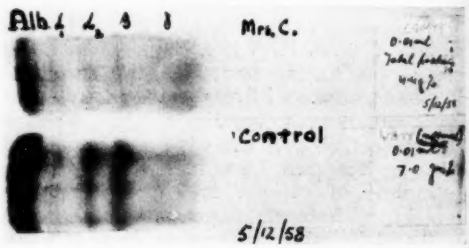


FIGURE I

Serum electrophoresis two days after operation, showing a lowering of albumin and of alpha-1, alpha-2 and beta globulin levels. The total protein content was 4·4 and 7·0 grammes per 100 ml. for the patient and control respectively

soon settled to normal. In summary, at this stage the only positive findings were marked hypoproteinæmia and an abnormal response to the liver function test as revealed by the " Bromsulphalein " retention test. In preparation for laparotomy, and because her hæmoglobin value was only 7·9 grammes per 100 ml., seven bottles of packed cells were transfused over several days, and on December 3 Dr. R. P. Melville performed a laparotomy. The caecum and colon appeared normal; the terminal part of the ileum was a little more congested than usual, and the serous coat showed slightly increased vascularity. There was no abnormality on palpation of the bowel wall. At the position of the Meckel's diverticulum an extension of the mesenteric fat covered the wall of the bowel, and here the number of subserosal vessels seemed to be markedly increased. A biopsy specimen of the wall of the small intestine was taken at this level. Biopsy specimens were then taken from small purplish patches (probably a hæmatoma) about four feet from the ileo-cæcal valve; specimens were also taken from a mesenteric lymph gland opposite the Meckel's area. The remainder of the small bowel appeared normal. The stomach was normal. The liver was enlarged, mottled and rather pale in colour. A biopsy specimen was taken from the edge of the left lobe. One specimen from the small bowel showed gross hæmorrhage into œdematous submucosa and mild cellular infiltration of the muscle coats with slight congestion of the

peritoneal surface. The other showed an acute ulcerative lesion situated in the mucosa, with gross œdema of the lamina propria and submucosa and infiltration by chronic and acute inflammatory cells (Figure II). The liver section was typical of prolonged protein deficiency. There was gross fatty infiltration (Figure III), most severe in the periportal regions.



FIGURE II
Section taken from lower part of ileum. See text

Multicellular cysts surrounded by the nuclei of the original cells and accompanied by mononuclear cell infiltration with occasional polymorphs were present in some places. In some small scattered areas liver-cell degeneration had proceeded to cell necrosis, and these small foci were surrounded by inflammatory cell infiltration. In addition to the foregoing features there were some microscopic areas of cellular aggregates, which appeared to be minute granulomas. However, no acid-fast bacilli could be seen on examination of numerous serial sections. The nature and pathogenesis of these minute granulomatous areas were obscure. The lymph nodes showed some follicular hyperplasia, with no other specific pathological changes. In summary, the pathological diagnosis suggested acute ileitis of unknown ætiology and severe liver damage.

After operation the patient developed a thrombosis in her left femoral vein and appeared to have a pulmonary embolus which was treated with anticoagulants. Six days after operation, the administration of prednisone was commenced in a dosage of 60 mg. for the first day, followed by 30 mg. per day for two days, and then 15 mg. per day thereafter. Then followed a stormy post-operative course, with diarrhœa still troublesome, some hæmorrhage from the suture line and infection in the wound, which was healing poorly. The wound condition slowly improved, despite the continuance of the prednisone therapy and with the help of various antibiotics. On January 21, 1959, after some seven weeks, it appeared intact. On February 2 a liver biopsy (Figure IV) was performed, and the report was as follows :

> Compared to the previous biopsy this liver biopsy shows a great improvement. There are now only minor abnormalities in the histological picture. There is no obvious fatty infiltration though some of the cells are slightly swollen and appear to show a minor degree of hydropic degeneration.

During this period of wound healing the diarrhœa appeared to decrease and only two further bouts occurred, the last in March. Her ankles were still œdematous. A serum protein estimation at this time showed the total protein content to be 5·9 grammes per 100 ml., and paper electrophoresis indicated that the albumin content was still slightly below normal. On March 26, the total protein content was 6·9 grammes per 100 ml., and the albumin band was now equally

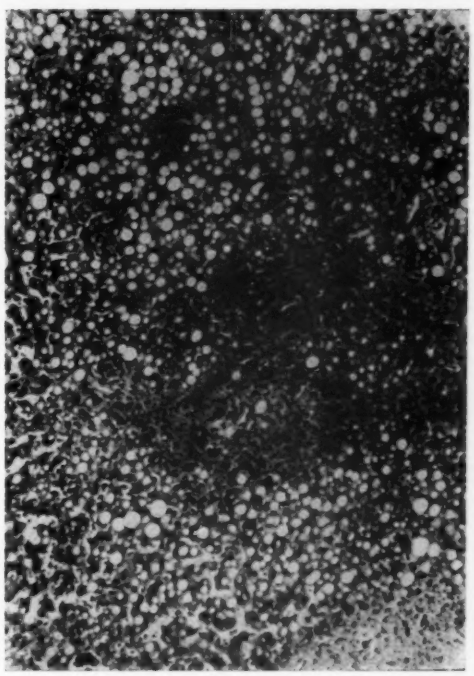


FIGURE III
Low-power view of liver, showing fatty infiltration

as dense as the control. There was some elevation of the alpha-1 and alpha-2 globulin levels. In March, the patient was in good health, with some slight pitting œdema of the ankles, but this was absent in the mornings. Her bowel actions were now virtually normal. On May 18, the total serum protein content

had risen to 7·3 grammes per 100 ml. At this time she stopped her prednisone tablets abruptly, on her own account, without any serious reaction, and when she was examined three weeks later for follow-up investigation she appeared well and the diarrhœa had not returned, so administration of the tablets was not recommenced. However, she appeared hypothyroid, and despite a high result to the radioactive iodine uptake test, thyroid therapy was commenced. She appeared to improve clinically over the next few months as far as her cerebration and general appearance were concerned. A liver biopsy performed on May 14, 1959, showed only some minor irregularities of the cells. Some of the parenchymal cells were slightly swollen, and in some areas there was definite fatty infiltration. In a few places there was a slight increase in the mononuclear cells lining the sinusoids, but the significance of this was uncertain, and the diagnosis was slight fatty infiltration of the liver. An ACTH test for assessing adrenal function was performed two months and three months respectively after the cessation of prednisone therapy, and on the second occasion the result was normal. On September 18, when she was free of diarrhœa and clinically well, with virtually normal serum protein levels, an investigation using radio-iodinated P.V.P. was undertaken, which will be described shortly. On October 30 the patient was readmitted to hospital for further radioactive studies using I^{131}-labelled albumin, the results of which are given below.

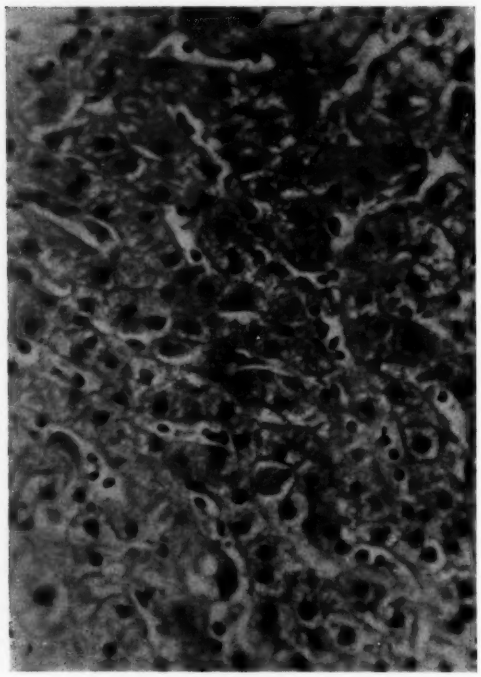


FIGURE IV
Liver biopsy, showing improvement in histological picture

After operation, the hæmoglobin value steadily rose, and became stabilized at about 12·8 grammes per 100 ml. The glucose tolerance curve was rather flat, the figures (milligrammes per 100 ml.) being as follows: fasting, 64; at half an hour, 82; at one hour, 110; at one and a half hours, 88; at two hours, 65. Shortly after her admission to the Unit, the prothrombin index was 100% and the serum alkaline phosphatase content was 22·5 K.A. units. The bromsulphthalein retention at this time was 11·3% at 45 minutes. The serum protein distribution is set out in Table I. An electrophoretogram on March 26 showed the albumin to be equally as dense as the control. The serum cholesterol content was 245 mg. per 100 ml., and the blood urea content was 65 mg. per 100 ml. A three-day fat-balance study was performed when the patient was clinically well, and no evidence of steatorrhœa was found. This was repeated, with the same result. An analysis of a twenty-four-hour specimen of urine for 5-hydroxyindole acetic acid gave a negative result.

I am indebted to Mr. K. N. Wynne, of the Special Unit, for the following notes on the radiochemical studies:

Methods.—I^{131}-labelled albumin (The Radiochemical Centre, Amersham, England) was used to determine albumin turnover according to the technique of Veall and Vetter (1958).

I^{131}-P.V.P. was obtained from Dr. R. S. Gordon (National Institutes of Health, Bethesda, Md., U.S.A.) through the agencies of the Commonwealth X-ray and Radium Laboratory, Melbourne.

TABLE I
Serum Protein Analyses

Date	Serum Protein Content (Grammes per 100 ml.)					
	Total	Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin
1958:						
September 20	3·7	1·59	—	—	—	—
November 19	4·4	*	*	*	*	*
December 3 (operation)	—	—	—	—	—	—
1959:						
January 9	5·9	Low	—	—	—	—
March 26	6·9	Normal	Raised	Raised	—	—
May 18	7·3	Normal	—	—	—	—
June 19	6·9	—	—	—	—	—
July 15	6·8	—	—	—	—	—
September 18	—	3·4	—	—	—	—

* See Figure I.

25 μc. of radioactivity was injected intravenously and a 96-hour period pooled fæcal collection instituted after the procedure of Gordon (1959).

Results.

Plasma Albumin—3·4 G./100 ml. (Normal Range 3·9–4·96 G./100 ml.).

Plasma Albumin Pool—1·5 G./Kg. (Normal Range 1·61–2·87 G./Kg.).

Mean Percentage Degradation Rate—29·7%/day.

Degradation Rate—0·46 G./day/Kg. (Normal Range 0·175–0·243 G./day/Kg.).

I^{131}-P.V.P. Results.

		Age	% Dose in Fæces
Patient ..	I.C. (F)[1] ..	60	2·9±0·4[2]
Controls ..	I.E. (F)[3] ..	50	1·1±0·3[2]
	J.L. (F)[4] ..	39	0·8±0·3[2]

Subsequent to the carrying out of the foregoing tests, we were informed that our particular shipment of I^{131}-P.V.P. contained approximately half its radioactivity in the form of inorganic iodide. In the absence of diarrhœa, this would have had the effect of reducing the fæcal excretion (in terms of the percentage of administered radioactivity) of this preparation when compared to others. Our finding of 2·9% may be regarded as even more significant.

DISCUSSION

This patient's diarrhœa appears to have caused a loss of plasma protein through her bowel, and to have led to such severe body depletion as to damage her liver and interfere with serum protein synthesis. This is suggested by the first liver biopsy, which showed virtually total replacement of all liver cells by fatty infiltration. Leg œdema developed, no doubt owing to the low albumin level in her serum (1·59 grammes per 100 ml.). The anæmia may well have been due to protein deprivation. Steroid therapy apparently induced a slow remission with healing of the liver damage, as evidenced by further biopsies. Associated with this was the quick improvement in serum protein levels.

Some months later, when she appeared clinically well, was no longer taking steroids, and had no further diarrhœa, an investigation was undertaken using radioactive iodine-labelled albumin and I^{131}-P.V.P. The former showed her to have an above-average rate of " degradation " of albumin. The I^{131}-P.V.P. test showed an excessive loss in the fæces, suggesting that even in the absence of diarrhœa she was losing an excessive amount of serum protein by this route.

[1] I.C., the patient who is the subject of the paper.
[2] Standard error.
[3] I.E. had radical mastectomy nine days previously.
[4] J.L. had a radical mastectomy the day before test began.

With the liver now healthy, it would appear that she is able to maintain an increased rate of synthesis to keep pace with her intestinal loss. Studies by Gordon *et alii* (1959) suggest that the liver can increase its synthesis of serum albumin to only twice the normal output in hypoalbuminæmic states, and any further liver damage may again possibly tip the scales against her and cause hypoproteinæmia again.

Both before and after the onset of her diarrhœa there was no lack of protein in the diet, nor was there any evidence of previous liver damage. The almost complete histological recovery of the liver is further evidence that the liver was not the prime cause of her hypoproteinæmia. A puzzling feature was the presence for over four weeks of fever which could not be explained. There was no leucocytosis, and all diagnostic and therapeutic attempts failed to elucidate it. Remission occurred just before steroids were used.

A case of protein-losing enteropathy which responded to steroids has also been reported by Gordon *et alii* (1959), in which it appeared to slow up the rate of decline of the serum albumin. However, this response was not elicited on a subsequent occasion.

Schwartz and Jarnum (1959) used I^{131}-P.V.P. on some normal subjects and on four patients with idiopathic hypoproteinæmia. The following modes of presentation occurred: œdema was present in all four cases; diffuse hypertrophic gastritis was present in two; small intestinal lesions were found in the other two.

In the present case, the result of the barium-meal X-ray examination was reported as normal, and at laparotomy minor non-specific changes only were found in the small bowel.

Reviewing a further 31 cases from the literature, Schwartz and Jarnum found gastrointestinal symptoms to be present in 14, mostly as diarrhœa, and at least three patients had giant hypertrophic gastritis.

It is probably incorrect to describe protein-losing gastro-enteropathy as a syndrome, as it may be an incidental finding in quite a large number of different bowel disorders. The most frequently noted one is giant hypertrophic gastritis, but gross protein loss has been recorded in steatorrhœa (Schwartz and Jarnum, 1959). Carcinoma of the stomach also causes protein loss (Gordon *et alii*, 1959). Holman *et alii* (1959) have described cases of disease of the small intestine producing the syndrome, and in some cases surgical excision has led to cure.

ACKNOWLEDGEMENT

My thanks are due to Dr. K. W. Starr, Honorary Director of the Special Unit, Prince of Wales Hospital, for permission to publish this case.

REFERENCES

ALBRIGHT, F., FORBES, A., BARTTER, F., REIFENSTEIN, E., BRYANT, D., COX, L., and DEMPSEY, E. (1950), "Studies on the Fate of Intravenously Administered Human Plasma Proteins in Idiopathic Hypoproteinæmia", "Symposium on Nutrition", Thomas, Springfield, Volume 2.

GORDON, R. S. (1959), "Exudative Enteropathy", *Lancet*, **1**, 325.

GORDON, R. S., BARTTER, F. C., and WALDMANN, T. (1959), "Idiopathic Hypoalbuminæmias", *Ann. intern. Med.*, **51**, 553.

HOLMAN, H., NICKEL, W. F., and SLEISENGER, M. H. (1959), "Hypoproteinæmia Antedating Intestinal Lesions and Possibly Due to Excessive Serum Protein Loss into the Intestine", *Amer. J. Med.*, **27**, 963.

SCHWARTZ, M., and JARNUM, S. (1959), "Gastrointestinal Protein Loss in Idiopathic (Hypercatabolic) Hypoproteinæmia", *Lancet*, **1**, 327.

SCHWARTZ, M., and THOMSEN, B. (1957), "Idiopathic and Hypercatabolichypoproteinæmia", *Brit. med. J.*, **1**, 14.

STEINFIELD, J., DAVIDSON, J., and GORDON, R. A. (1957), "Mechanism for Hypoalbuminæmia in Patients with Ulcerative Colitis and Regional Ileitis", *J. clin. Invest.*, **36**, 931.

VEALL, N., and VETTER, H. (1958), "Radioisotope Techniques in Clinical Research and Diagnosis", Butterworth, London.

ADDENDUM

A follow-up estimation of serum proteins and electrophoresis was performed on July 11, 1960, 22 months after the patient's admission to the Unit. The total serum protein content was 7·7 grammes per 100 ml., and the pattern was normal. The patient remains in good health.

SERUM VITAMIN B_{12} LEVELS IN CHRONIC ATROPHIC GASTRITIS[1]

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SUMMARY

Thirteen cases of severe chronic atrophic gastritis were studied over a period ranging from five to 12 years. Diagnosis was established by a history of flatulent dyspepsia, depleted acid secretion and serial gastric biopsies using the suction biopsy tube. There was no evidence of peptic ulcer or cancer.

During the last three years of the study serial assays were made of the serum vitamin B_{12} levels in these cases. In the majority there was a fall in the level. Also, the mean level was lower than that in a control group in which normal acid secretion was found on histamine stimulation. Absorption studies were made with ^{58}Co labelled vitamin B_{12}.

These studies indicated the following three clinical states: (i) *pre-pernicious anæmia*, with serum vitamin B_{12} levels between 100 and 250 μμg. per millilitre and normoblastic bone marrow, but no symptoms or perhaps minimal weakness; (ii) *latent pernicious anæmia*, with a serum vitamin B_{12} level less than 100 μμg. per millilitre, but still a normoblastic bone marrow, and either no symptoms or, at the most, minimal weakness; (iii) *overt pernicious anæmia*, with a serum vitamin B_{12} level of less than 100 μμg. per millilitre and the classical clinical and bone-marrow changes of pernicious anæmia and/or subacute combined degeneration of the spinal cord.

The findings after a histamine test meal and on gastric biopsy and particularly the serum vitamin B_{12} level are of considerable value to the physician who suspects prepernicious anæmia, latent pernicious anæmia or the early manifestations of overt pernicious anæmia or subacute combined degeneration of the cord. He may thus save the patient from the severe forms of the disease.

STUDIES of the gastric mucosa began in our hospital in 1948 with the development of the suction biopsy tube (Wood *et alii*, 1949*a*). Early observations indicated that in classical pernicious anæmia the gastric lesion was that of severe atrophy, the inflammatory changes typical of gastritis being absent or minimal (Motteram, 1951; Doig and Wood, 1950). It was postulated then that this state of the gastric mucosa was due to primary atrophy, and was not a sequela of gastritis. One of our patients, a male, aged 65 years, with gastric atrophy and histamine-fast achlorhydria, was followed for five years before he developed overt pernicious anæmia and subacute combined degeneration of the cord, responding to treatment with vitamin B_{12} (Robertson *et alii*, 1955). The result of the Schilling test was positive. Gastric atrophy may have followed atrophic gastritis, as he had suffered intermittent flatulent dyspepsia for forty years.

Our further observations over the past ten years revealed that in some cases of pernicious anæmia the inflammatory changes of active chronic gastritis were present in addition to the atrophy (Joske *et alii*, 1955; Wood and Taft, 1958). Similar observations have been recorded by Magnus (1958).

PATIENTS SELECTED FOR STUDY

In order to determine whether some patients with pronounced chronic atrophic gastritis would develop overt pernicious anæmia or subacute combined degeneration of the cord, serial observations were made in 13 cases of chronic atrophic gastritis. They were chosen at random and studied for five to twelve years. When first selected, all the 13 patients had atrophic gastritis on gastric biopsy, achlorhydria or low secretion of acid, and no evidence of ulcer or cancer. These findings were present throughout the study.

When first examined, most patients gave a history of five or more years of intermittent flatulent dyspepsia typical of chronic atrophic gastritis. No patient had changes in the blood or nervous system indicative of pernicious anæmia or subacute combined degeneration of the cord.

[1] Received on May 25, 1960.

[2] Head of the Clinical Research Unit, working with the aid of a grant from the National Health and Medical Research Council of Australia.

[3] Clinical Pathologist.

[4] Assistant Clinical Pathologist.

[5] Bacteriologist.

The serial observations on this group included a clinical history and examination, gastric biopsy, a histamine test meal, X-ray examination with a barium meal, cytological examination of the peripheral blood and bone marrow, vitamin B_{12} assay of the serum, and vitamin B_{12} absorption studies using ^{58}Cobalt-labelled vitamin B_{12}.

No patient received vitamin B_{12} therapy before or during the period of observation. In two cases the study was discontinued when B_{12} therapy was given, in Case I because of a low serum B_{12} level and severe angina, and in Case II owing to a misunderstanding by the patient's country doctor.

METHODS

Gastric biopsy was performed with the flexible suction gastric biopsy tube designed in our Clinical Research Unit (Wood *et alii*, 1949*a*; Wood and Taft, 1958).

The histamine test meal examination was carried out over a period of 90 minutes after a standard dose of 0·9 mg. of histamine acid phosphate. In subjects showing a low serum level of vitamin B_{12}, the maximum secretory response described by Kay (1953) was tested.

The serum vitamin B_{12} level was estimated by the method of Ross (1952) using *Euglena gracilis Z* strain and the basal medium of Hutner *et alii* (1956). Absorption of ^{58}Cobalt-labelled (^{58}Co labelled) vitamin B_{12} was measured over a period of six to eight days by the fæcal excretion technique of Heinle and his co-workers (1952). An intramuscular injection of "Carbachol"[1] (carbamylcholine chloride) was given immediately after the oral dose of radioactive vitamin B_{12}, as Mollin *et alii* (1957) found that this increased the absorption in control (normal) subjects, and to a lesser degree in cases of pernicious anæmia. It thus enhanced the value of the test as a means of diagnosing true pernicious anæmia. Five patients with normal fæcal excretion were retested without carbachol.

With the exception of Case I, in which latent pernicious anæmia requiring vitamin B_{12} therapy developed, the urinary excretion (Schilling) test was not used, as it entailed the parenteral administration of a large flushing dose of vitamin B_{12}, thus disturbing the natural levels of vitamin B_{12} in the serum (Schilling, 1953).

RESULTS

The 13 patients with chronic atrophic gastritis included in the present study comprised five males and eight females whose mean age was 62 years (range 47 to 77). They were observed for periods ranging from five to 12 years (mean 10 years), and all suffered from intermittent flatulent dyspepsia. This discomfort was usually mild in degree, but three had attacks of pain of moderate severity. Three had no detectable disease other than gastritis; three had suffered from gall-bladder disease with gall-stones which had been successfully treated by cholecystectomy, two from angina pectoris, two from osteoarthritis, one from hypertension, one from rheumatoid arthritis and one from recurrent pyelitis.

The blood pressure levels were not remarkable for the age group, ranging from 110/65 to 180/115 (mean 154/89) mm. Hg.

Of the 13 patients, three occasionally had pronounced discomfort in the epigastrium; but this was diffuse and not of the severity of chronic ulcer in relapse or of pancreatitis. In five there was mild epigastric tenderness and in three minor enlargement of the liver. Neither X-ray nor gastroscopic examination revealed any evidence of ulcer or carcinoma. Relevant studies of the gall-bladder (*vide supra*) and pancreas also gave negative results.

No patient in the series suffered from pronounced malnutrition either before or during the study. When the 13 patients were selected, 10 were assessed as having an adequate diet, and three as having a diet with a minor deficiency of protein and vitamins. However, during the study all took a full diet adequate in animal protein and dairy produce; there were no vegetarians, as described by Harrison *et alii* (1956). No patient had liver disease (Rachmilewitz *et alii*, 1956; Cowling and Mackay, 1959) or was addicted to alcohol; nine patients took no alcohol or only an occasional drink, four took regular small amounts of beer and an adequate diet.

Biochemical Tests

Throughout the study there was no pronounced abnormality of the serum albumin, globulin or gamma globulin contents. The mean values at the end of the study were: albumin, 4·4 grammes per 100 ml.; globulin, 2·6 grammes per 100 ml.; gamma globulin, 1·0 gramme per 100 ml. There was no pronounced albuminuria or elevation of the blood urea level (range 24 to 56 mg. per 100 ml.). There was no chronic diarrhœa or excess fæcal fat. Serum lipides and cholesterol levels were normal. Tests of the fæces for occult blood gave negative results in 10 cases and an occasional positive result in three.

Test Meal.—Serial histamine test meals in the 13 cases showed achlorhydria on at least three occasions in all, the mean number of

[1] British Drug Houses.

TABLE I

The Findings in 13 Cases of Atrophic Gastritis. The Age Stated was at the End of the Study. The Serum Vitamin B_{12} Levels were Estimated over a Mean Period of Three Years

Case Number	Sex	Age (Years)	Period of Observation (Years)	Histamine Test Meal (Units of Free Hydrochloric Acid)			Serum Vitamin B_{12} Level (Micromicrogrammes per Millilitre)			Hæmoglobin Value (Grammes per 100 ml.)			Mean Corpuscular Volume (c.μ.): End
				Beginning	End	Range	Beginning	End	Range	Beginning	End	Range	
I	M.	74	9	0	0	0	162	50	50–162	15·0	15·3	14·2–15·3	90
II	F.	53	11	0	0	0	238	122	122–238	6·6	13·7	6·6–15·0	91
III	F.	66	11	15	0	0–15	300	180	107–300	12·8	14·3	12·1–14·3	89
IV	F.	74	12	0	0	0–40	277	208	114–236	12·4	13·0	12·4–16·0	100
V	M.	63	11	5	0	0–22	300	230	230–300	13·6	14·0	13·6–16·1	95
VI	F.	76	10	0	0	0	562	300	300–362	13·3	10·9	9·2–13·3	85
VII	F.	53	10	0	0	0	325	300	240–362	16·0	14·8	14·8–16·0	87
VIII	M.	66	5	0	0	0	520	333	333–520	15·0	15·0	14·9–15·0	98
IX	F.	48	11	0	12	0–30	330	362	245–624	14·0	13·1	12·1–14·9	89
X	M.	49	10	0	0	0–10	580	370	303–580	16·2	17·5	16·2–17·5	86
XI	F.	75	5	0	0	0	400	425	290–425	14·8	14·2	13·6–14·8	85
XII	M.	47	8	0	0	0	278	440	225–440	16·5	14·3	13·4–16·5	90
XIII	F.	56	12	0	0	0–22	360	460	245–624	13·7	14·2	13·3–14·6	82

occasions being five (range three to seven). Seven patients had achlorhydria throughout; early in the study six had minor elevations of free gastric acid above zero, the average rise being to 15 units of free acid. Twelve patients had achlorhydria at the end of the study, and the remaining one 12 units (Table I). Five patients with lower serum levels of vitamin B_{12} showed achlorhydria after maximum histamine stimulation (Kay, 1953).

Gastric Biopsy

Sixty-five gastric biopsies were performed on the 13 patients. The number of biopsies per patient ranged from two to 10, and the period over which they were taken ranged from five to 12 years (mean, nine).

All the 65 biopsies showed infiltration of the lamina propria with lymphocytes and plasma cells, and a limited number of polymorphs. This infiltration was pronounced in both the superficial and the deep layers of the lamina propria in 60 specimens, and mainly in the superficial layer in five.

In all specimens there was pronounced atrophy of the acid and pepsinogen secreting cells. In 41 specimens this was severe, with only a few of these cells remaining, and in the remaining 24 atrophy was patchy and less pronounced.

The degree of atrophy was greater as the study progressed, and finally the biopsy on all patients showed severe atrophic gastritis. In three patients with severe atrophy the cellular infiltration was less pronounced, the appearance approaching gastric atrophy (Motteram, 1951).

Peripheral Blood and Bone-Marrow Examination

Examination of the peripheral blood in the 13 cases showed no pronounced deviation from the normal except in two, in which microcytic hypochromic anæmia was present, the initial hæmoglobin values being 6·6 grammes per 100 ml. (Case II) and 9·2 grammes per 100 ml. (Case VI). Both patients responded to courses of iron therapy, but tended to relapse. The mean hæmoglobin value of the group over the years was 13·4 grammes per 100 ml., being 13·5 grammes per 100 ml. (range 6·6 to 16·5) at the beginning of the study and 14·5 grammes per 100 ml. (range 10·9 to 16·0) at the end (Table I). Other findings at the end were: red cell count, mean 4,700,000 per cubic millimetre (range, 3·5 to 5·8); mean corpuscular volume, mean 88 c.μ. (range, 82 to 95); leucocyte count, mean 7600 per cubic millimetre (range, 5200 to 10,200). Biopsies of the bone marrow were performed on five patients with the lowest serum vitamin B_{12} levels, and none showed a megaloblastic change indicative of pernicious anæmia.

Examination of the Nervous System

None of the 13 patients developed overt subacute combined degeneration of the cord during the period of study. In nine cases, examination of the nervous system showed no abnormality throughout. In four there was some diminution of the knee and ankle jerks; however, no patient complained of paræsthesia, and none had a Babinski response or reduction in objective sensation. None had pronounced muscular weakness on clinical testing, but six complained of slowly increasing failure of mental acuity and some general weakness. These were of minor degree and attributed to the aging process; lowered vitamin B_{12} levels may have contributed in some patients.

Serum Vitamin B_{12} Levels

Serial estimations were made of the serum vitamin B_{12} levels of the 13 members of the group during the last three years of the twelve-year study. Over a period ranging from 20 to 36 months (mean 30), a total of 64 estimations were made, and the mean level was 281 μμg. per millilitre (range, 50 to 624). Although there were fluctuations in the level, nine of the 13 showed a fall (see Table I and Figure I). At

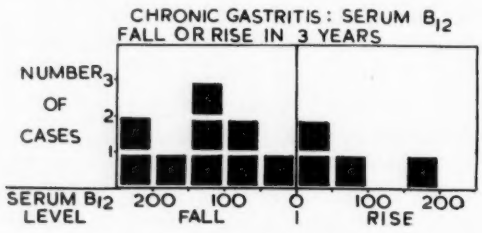


Figure I

The rise or fall in the serum levels of vitamin B_{12} (micromicrogrammes per millilitre) in 13 cases of atrophic gastritis studied over a mean period of three years. The levels at the beginning and end of the study were used in recording this case distribution

the beginning of the observations, the mean serum vitamin B_{12} level was 356 μμg. per millilitre (range, 162 to 580), and at the end 291 μμg. per millilitre (range, 50 to 460) (Figure II).

As a control, 13 individuals were selected at random, except that they were paired for sex and age with the 13 patients in the test series; none suffered from gastro-intestinal disease, nervous disease or alcoholism. Although these controls were not subjected to gastric biopsy, they were all found to have normal acid secretion on examination with a histamine test meal; this excluded any pronounced diffuse change in the gastric mucosa (Wood *et alii*, 1949*b*; Joske *et alii*, 1955). These controls had a mean serum vitamin B_{12} level of 458 μμg. per millilitre (range, 245 to 800), an increase over the final levels in the test series with chronic atrophic gastritis (mean 291) (Figure II). Spray and Witts (1958) examined 123 normal individuals, and found a range of 150 to 1000 μμg. per millilitre with a mean of 450 μμg. per millilitre. Moreover, they found that 90% of 94 patients with untreated overt pernicious anæmia had levels of 100 μμg. per millilitre or less.

Absorption of ^{58}Co-labelled Vitamin B_{12}

Seven patients in the series with lower serum levels of vitamin B_{12} were submitted to fæcal excretion tests, "Carbachol" being used as a stimulus to the secretion of intrinsic factor. The result was normal in six cases, the percentage absorption of the oral dose being 48, 50, 51, 54, 56 and 60 (normal range, 30% to 90% of a 1·0 μg. dose of ^{58}Co B_{12}). The remaining patient (Case I), who had the lowest serum level of vitamin B_{12} (50 μμg. per millilitre), had impaired absorption, absorbing only 19% of the oral dose. This was increased to the normal level of 40% when intrinsic factor was added to the oral dose of ^{58}Co-labelled vitamin B_{12}. Also, the Schilling test revealed impaired absorption. Radioactivity amounting to 6% of an oral dose of 1·0 μg. of ^{58}Co B_{12} was excreted in the urine over 48 hours (normal range, 10% to 30%).

It was considered that the finding of normal absorption in six of the seven patients with atrophic gastritis and low or moderately low serum levels of vitamin B_{12} might have been due to the "Carbachol" causing an excessive and unnatural stimulus to the secretion of

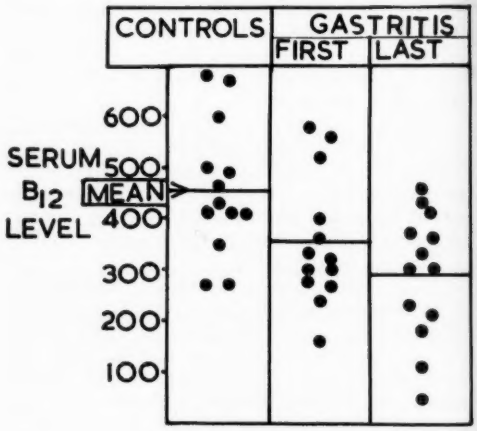


Figure II

The serum levels of vitamin B_{12} (micromicrogrammes per millilitre) in 13 patients with atrophic gastritis compared with 13 controls, matched for age and sex but having normal acid secretion to histamine stimulation. The test series was studied over a mean period of three years, and the levels at the beginning and the end of the study are shown

intrinsic factor. The test would thus fail to reveal minor degrees of deficiency of the intrinsic factor, such as might occur in severe atrophic gastritis. Therefore, in five of these cases the fæcal excretion test was repeated without "Carbachol" stimulation; but the results still indicated normal percentage absorption (50%, 80%, 54%, 42% and 61% respectively).

Records of Six Patients with Lower Serum Levels of Vitamin B_{12}

The following brief case records are representative of the test series, and have been placed in order of depletion of the patients' final serum vitamin B_{12} level. Case I is notable, as this patient's level fell to the pernicious anæmia range.



FIGURE III

Case I: chronic atrophic gastritis. Gastric biopsy made in 1950 from a male, aged 66 years, who had suffered flatulent dyspepsia for 10 years. He had achlorhydria, but no change in the peripheral blood or bone marrow. The surface epithelium is normal, but there is intestinal metaplasia in one pit. There is almost complete atrophy of the acid and pepsinogen secreting cells, with pronounced infiltration of the lamina propria with inflammatory cells. Under higher magnification these are shown to be mostly lymphocytes, with a few plasma cells and polymorphs. A lymph follicle is present on the left. There is some splintering of the muscularis mucosæ with inflammatory cells. (Hæmatoxylin and eosin stain. (×100)

CASE I.—When first examined in 1950, a male, aged 66 years, had suffered from minor flatulent dyspepsia for 10 years. Achlorhydria to maximum histamine stimulation and severe atrophic gastritis on gastric biopsy were found. In 1957, he developed increasing angina of effort with many ventricular extrasystoles. Serial gastric biopsies in 1950, 1955, 1959 and 1960 constantly showed atrophic gastritis with almost complete atrophy of the acid and pepsin secreting cells, limited infiltration of the tunica propria with lymphocytes and plasma cells and some fibrosis (Figures III and IV). Repeated examinations of the peripheral blood and bone marrow provided no evidence of pernicious anæmia; his hæmoglobin value was 15·3 grammes per 100 ml., the erythrocytes numbered 5,000,000 per cubic millimetre, and the leucocytes 8000 per cubic millilitre, the mean corpuscular volume was 90 c.μ., the mean corpuscular hæmoglobin was 30 m.μg., and the mean corpuscular hæmoglobin concentration was 33%. Examination of the nervous system showed diminution of the deep reflexes, but no overt subacute combined degeneration of the cord. The serum vitamin B_{12} level was low (162 μμg. per millilitre) when it was first examined in 1957, and in 1959 it fell to the pernicious anæmia range (50 μμg. per millilitre), as shown in Figure V. A fæcal excretion test with ^{58}Co labelled vitamin B_{12} and "Carbachol" injection gave an abnormal result (absorption of 19% of a dose of 1·0 μg.) and the result was restored to normal (40% absorption) when the test was repeated with intrinsic factor. The response to the Schilling test of urinary excretion was abnormal (6·2% of a dose of 1·0 μg.). There was no steatorrhœa, and a jejunal biopsy revealed no abnormality (Ralston *et alii*, 1960).

In October, 1959, he was given vitamin B_{12} injections in full dosage because of the increasing severity of the anginal pain, and the low serum level of vitamin B_{12} and its impaired absorption. Within two weeks there was considerable lessening of the anginal pain with a gain in strength. He remained well until last seen in February, 1960.

CASE II.—A housewife, aged 53 years, had suffered from recurrent flatulent dyspepsia and microcytic hypochromic anæmia for 17 years when she was admitted to the Unit for study in 1949.

The dyspepsia continued, but the anæmia was relieved by oral iron therapy, the hæmoglobin value rising from 6·6 to 15·0 grammes per 100 ml., with relapse when iron was omitted. There was no enlargement of the liver or spleen. Occult blood was absent from the stools. There was histamine-fast achlorhydria, and gastric biopsies in 1949 and 1957 revealed severe atrophic gastritis (Figure VI). Estimations of the serum vitamin B_{12} level between 1957 and 1959 showed a progressive fall (238, 190, 140 and 122 μμg. per millilitre), as shown in Figure V. However, examination of the peripheral blood revealed no evidence of pernicious anæmia, and there were no manifestations of subacute combined degeneration of the spinal cord. The response to the fæcal excretion test of labelled

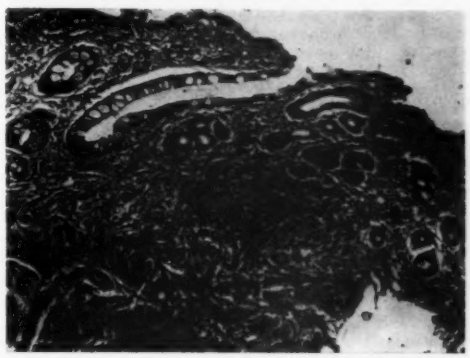


FIGURE IV

Case I: Chronic atrophic gastritis approaching gastric atrophy. Gastric biopsy obtained nine years after that shown in Figure III. The serum vitamin B_{12} level had fallen from 162 to 50 μμg. per millilitre (Figure V), but there was still no change in the peripheral blood or bone marrow. There is atrophy of the surface epithelium, with some intestinal metaplasia extending into the pits. The specific glands have disappeared, and there is less pronounced inflammatory reaction in the tunica propria. The appearance now approaches gastric atrophy. (Hæmatoxylin and eosin stain. ×100)

vitamin B_{12} was within the normal range with "Carbachol" stimulation (52% absorption) and without (54% absorption). In July, 1959, the patient was inadvertently given a course of vitamin B_{12} injections by her country doctor; these caused a prolonged rise in the serum level.

CASE III.—For 11 years a housewife, aged 66 years, was studied because of diffuse epigastric discomfort, achlorhydria to maximum histamine stimulation and atrophic gastritis (biopsies 1949, 1950, 1951, 1953, 1957

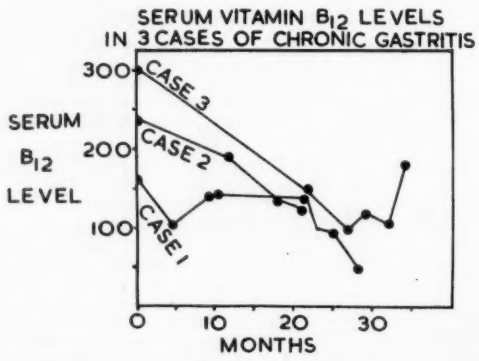


FIGURE V

Serum vitamin B_{12} levels (micromicrogrammes per millilitre) in three patients with chronic atrophic gastritis, with low levels. None showed overt pernicious anæmia or subacute combined degeneration of the cord

and 1960) (Figure VII). The Wassermann reaction was weakly positive, but there were no overt signs of syphilis; after penicillin therapy the reaction became negative. Examination of the peripheral blood and bone marrow revealed no evidence of pernicious anæmia. There was no evidence of subacute combined degeneration of the cord. Serial estimations of the serum vitamin B_{12} level gave values of 300, 137, 148, 100, 107 and 180 μμg. per millilitre (Figure V). Fæcal excretion tests gave normal results with "Carbachol" stimulation (50% and 42% absorption) and without (83% absorption).

CASE VII. A housewife, aged 54 years, was studied for 11 years. In 1949 a laparotomy was performed for suspected carcinoma of the stomach, but no carcinoma or ulcer was found. She continued to suffer moderately severe bouts of flatulent dyspepsia, with epigastric tenderness and some atrophy of the tongue. Serial gastric biopsies (1949, 1953, 1954, 1957 and 1959) revealed severe atrophic gastritis. There was constant achlorhydria to histamine stimulation. Examination of the peripheral blood and nervous system showed neither pernicious anæmia nor subacute combined degeneration of the cord. Serum vitamin B_{12} levels were not reduced, ranging between 300 and 400 μμg. per millilitre (Table I). The result of the fæcal excretion test with labelled vitamin B_{12} and "Carbachol" stimulation was normal (56% absorption).

CASE XII. A carpenter, aged 47 years, was studied for nine years. He suffered occasionally from epigastric discomfort. There was achlorhydria to histamine in 1951, 1952, 1954 and 1959 (maximum response). Gastric biopsies in 1952, 1954, 1957 and 1959 showed severe atrophic gastritis approaching gastric atrophy. The findings on jejunal biopsy were normal. The findings in the peripheral blood and bone marrow remained within normal limits. There was no abnormality of the nervous system. Serial serum vitamin B_{12} levels between 1957 and 1959 were normal, ranging between 225 and 330 μμg. per millilitre (Table I). Fæcal excretion of labelled vitamin B_{12} was normal with "Carbachol" stimulation (48% absorption) and without (52% absorption).

CASE XIII.—A housewife, aged 56 years, was studied for 11 years because of recurrent nausea, anorexia and abdominal discomfort. There was epigastric tenderness. Serial histamine test meal examinations showed achlorhydria on most occasions; but there was some free acid (22 units) in 1954 after the oral administration of penicillin. Gastric biopsies (1949, 1951, 1953, 1954 and 1960) revealed moderate atrophic gastritis progressing to a severe degree. There was no evidence of pernicious anæmia or subacute combined degeneration of the cord. Between 1957 and 1960, serum vitamin B_{12} levels fluctuated within normal limits (360, 624, 300, 245 and 448 μμg. per millilitre) (Table I). The result of the fæcal excretion test was normal with "Carbachol" stimulation (54% absorption) and without (43% absorption).

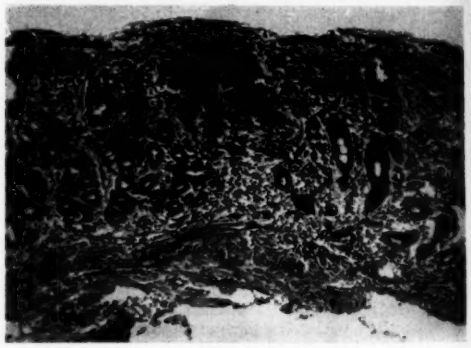


FIGURE VI

Case II: chronic atrophic gastritis. Gastric biopsy from a female, aged 53 years, with prolonged flatulent dyspepsia. There was achlorhydria and hypochromic anæmia, but no evidence of pernicious anæmia in the peripheral blood or bone marrow. The serum vitamin B_{12} level was 238 μμg. per millilitre, and later fell to 122 μμg. per millilitre (Figure V). This biopsy, obtained in 1957, shows the epithelium is low, with some shedding. There is considerable atrophy of the specific glands, and extensive infiltration of the lamina propria with inflammatory cells, shown under higher magnification to be mostly lymphocytes and polymorphs with occasional plasma cells. (Hæmatoxylin and eosin stain. ×100)

DISCUSSION

These studies, and those of others (Mollin, 1959), indicate that in progressive atrophic gastritis, which occasionally ends in gastric atrophy (Wood and Taft, 1958), there may be a slow reduction in intrinsic factor production, failure of B_{12} absorption and depletion of B_{12} stores in the liver. This causes a gradual fall

in both the serum vitamin B_{12} level and the supply of vitamin B_{12} to the tissues, including the hæmatopoietic and nervous systems.

Thus a patient with prepernicious anæmia, with moderately low serum levels of vitamin B_{12} (100 to 250 μμg. per millilitre) and perhaps some minor loss of mental acuity and muscular weakness, but no changes in the bone marrow,

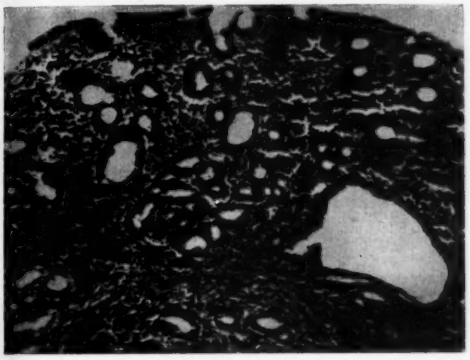


FIGURE VII

Case III: chronic atrophic gastritis. Gastric biopsy from a female, aged 66 years, with epigastric discomfort, achlorhydria and lowered serum vitamin B_{12} level (Figure V), but no abnormality in the peripheral blood or bone marrow. Six gastric biopsies made between 1949 and 1960 showed atrophic gastritis. This biopsy, obtained in 1960, is similar to that obtained in 1949, except that atrophy is more pronounced. The surface epithelium is flattened. There is cystic dilatation of the pits and gross atrophy of the acid and pepsinogen secreting cells. The tunica propria is densely infiltrated with inflammatory cells, shown under higher magnification to be mostly lymphocytes and plasma cells with a few polymorphs. (Hæmatoxylin and eosin stain. ×100)

may pass on to latent pernicious anæmia, with a serum vitamin B_{12} level less than 100 μμg. per millilitre and perhaps abnormal results on urinary (Schilling) and fæcal excretion tests, but still no classical clinical signs or change in the bone marrow; and perhaps the patient finally may pass on to overt pernicious anæmia, with the classical changes in the peripheral blood, bone marrow and nervous system (Table II).

In the present twelve-year survey there were 13 patients with proven chronic atrophic gastritis, having few remaining acid and pepsin secreting cells in their gastric mucosa on repeated gastric biopsies, and depleted acid secretion to histamine stimulation. During the last three years of the survey, estimations of their serum vitamin B_{12} concentrations revealed that some patients had concentrations in the lower levels of the "normal" range. There were fluctuations in the concentration, but over the period of observation nine of the 13 patients were found to have a fall. Moreover, when compared to a control group, paired for age and sex but having normal gastric secretion, this test group had a lower mean serum level of B_{12}, especially at the end of the study.

A previous report from this hospital (Robertson *et alii*, 1955) described the case of a patient with prepernicious anæmia who, after a lapse of five years, developed overt pernicious anæmia and subacute combined degeneration of the cord.

In the present series, an elderly male (Case I) with prepernicious anæmia developed latent pernicious anæmia. He had severe atrophic gastritis with histamine-fast achlorhydria, and the serum vitamin B_{12} level fell from 162 to 50 μμg. per millilitre, a concentration within

TABLE II

The Progress of Prepernicious Anæmia to Latent Pernicious Anæmia and Thence to Overt Pernicious Anæmia, with the Accompanying Findings

Clinical State	Symptoms	Serum Vitamin B_{12} Level (Micromicrogrammes per Millilitre)	Results of Fæcal and Urinary Excretion Tests with Labelled Vitamin B_{12}	Bone Marrow	Result of Histamine Test Meal Examination	Gastric Biopsy Findings
Prepernicious anæmia	Usually, nil Rarely, minor loss of mental acuity and muscular weakness	100–250	Normal or near normal	Normal	Achlorhydria or trace of free acid	Severe atrophic gastritis or gastric atrophy
Latent pernicious anæmia	Usually, nil Rarely, minor loss of mental acuity and muscular weakness	Less than 100	Abnormal	Normal	Achlorhydria	Severe atrophic gastritis or gastric atrophy
Overt pernicious anæmia and/or subacute combined degeneration of the spinal cord	Classical changes of pernicious anæmia and/or subacute combined degeneration of the spinal cord	Less than 100	Abnormal	Megaloblastic	Achlorhydria	Severe atrophic gastritis or gastric atrophy

the pernicious anæmia range. He also gave an abnormal response to the fæcal excretion test with ^{58}Co labelled vitamin B_{12}, which was restored to normal by the addition of intrinsic factor, and an abnormal response to the urinary excretion (Schilling) test. However, his peripheral blood and bone marrow were normal. He had some general muscular weakness attributed to old age, but no signs of overt pernicious anæmia or subacute combined degeneration of the cord. However, injections of vitamin B_{12} were given, as he suffered increasingly severe angina pectoris, which had failed to respond to routine measures. He stated that his condition improved.

At the end of the present study, four other patients were considered to be in the prepernicious anæmia stage, with reduced serum vitamin B_{12} levels ranging from 122 to 230 μμg. per millilitre. Although levels over 100 μμg. per millilitre are above the generally accepted pathological range, this may not be so, for although these patients showed no evidence of classical pernicious anæmia or subacute combined degeneration of the cord, some complained of weakness and loss of mental acuity. These symptoms were of minor degree, and usually were attributed to the aging process.

The presence of prepernicious anæmia may be suspected in patients with chronic flatulent dyspepsia, impaired acid secretion, gross atrophic gastritis on gastric biopsy and, most valuable of all, a depleted and falling serum level of vitamin B_{12}. However, in the present series the estimation of absorption of vitamin B_{12} from the fæces did not reveal impaired absorption, even when "Carbachol" stimulation was omitted. In a similar series, Mollin (1959) found that there was normal absorption with "Carbachol" stimulation, but deficient absorption when this was omitted. In their earlier studies, Mollin and Baker (1955) reported that some patients with "early" pernicious anæmia had some remaining vitamin B_{12} absorption.

In a study of the age factor, Halsted *et alii* (1959) found that with advancing age there was no significant fall in the serum vitamin B_{12} level in healthy individuals, but there was a fall when there was impaired gastric secretion of intrinsic factor. Joske and his colleagues from our hospital (1955) concluded that, although chronic atrophic gastritis was not an invariable finding in the elderly, it was more frequently found as age progressed. Siurala and Nyberg (1957) reported an inverse relationship between the severity of gastritis and the absorption of vitamin B_{12}, as measured by the urinary excretion (Schilling) test.

Thus some of the early and unwelcome symptoms of the aging process may occasionally prove to be the premonitory signs of the vitamin B_{12} deficiency—prepernicious anæmia. Most patients with prepernicious anæmia progress no further. A few may slowly pass on to latent pernicious anæmia. Now overt pernicious anæmia with its crippling changes in the blood or nervous system may be close at hand or may be delayed for a year or more (Robertson *et alii*, 1955).

It falls to the lot of the physician of today to recognize this sequence, and to examine the vitamin B_{12} level in the serum. This will enable him to administer vitamin B_{12} before severe symptoms of vitamin B_{12} deficiency become manifest.

ACKNOWLEDGEMENTS

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REFERENCES

COWLING, D. C., and MACKAY, I. R. (1959), "Serum Vitamin B_{12} Levels in Liver Disease", *Med. J. Aust.*, **2**, 558.

DOIG, R. K., and WOOD, I. J. (1950), "Gastric Biopsy in Pernicious Anæmia", *Med. J. Aust.*, **2**, 565.

HALSTED, J. A., CARROLL, J., and RUBERT, S. (1959), "Serum and Tissue Concentration of Vitamin B_{12} in Certain Pathological States", *New Engl. J. Med.*, **260**, 575.

HARRISON, R. J., BOOTH, C. C., and MOLLIN, D. L. (1956), "Vitamin B_{12} Deficiency due to Defective Diet", *Lancet*, **1**, 727.

HEINLE, R. W., WELCH, A. D., SCHARF, V., MEACHAM, G. C., and PRUSOFF, W. H. (1952), "Studies of Excretion (and Absorption) of Co^{60}-Labelled Vitamin B_{12} in Pernicious Anæmia", *Trans. Ass. Amer. Phycns.*, **65**, 214.

HUTNER, S. H., BACH, M. K., and ROSS, G. I. M. (1956), "A Sugar-Containing Basal Medium for Vitamin B_{12}-Assay with Euglena: Application to Body Fluids", *J. Protozool.*, **3**, 101.

JOSKE, R. A., FINCKH, E. S., and WOOD, I. J. (1955), "Gastric Biopsy: A Study of 1,000 Consecutive Successful Biopsies", *Quart. J. Med.* (N.S.), **24**, 269.

KAY, A. W. (1953), "Effect of Large Dose of Histamine on Gastric Secretion of HCl: An Augmented Histamine Test", *Brit. med. J.*, **2**, 77.

MAGNUS, H. A. (1958), "A Re-Assessment of the Gastric Lesion in Pernicious Anæmia", *J. clin. Path.*, **11**, 289.

MOLLIN, D. L. (1959), "Radioactive Vitamin B_{12} in the Study of Blood Diseases", *Brit. med. Bull.*, **15**, 8.

MOLLIN, D. L., and BAKER, S. J. (1955), "The Absorption and Secretion of Vitamin B_{12} in Man", *Symp. Biochem. Soc. No.* 13, p. 52.

MOLLIN, D. L., BOOTH, C. C., and BAKER, S. J. (1957), "The Absorption of Vitamin B_{12} in Control Subjects in Addisonian Pernicious Anæmia and in the Malabsorption Syndrome", *J. Haematol.*, **3**, 412.

MOTTERAM, R. (1951), "A Biopsy Study of Chronic Gastritis and Gastric Atrophy", *J. Path. Bact.*, **63**, 389.

RACHMILEWITZ, M., ARONOVITCH, J., and GROSSOWICZ, N. (1956), "Serum Concentration of Vitamin B_{12} in Acute and Chronic Liver Disease", *J. Lab. clin. Med.*, **48**, 339.

RALSTON, M., WOOD, I. J., and HUGHES, A. (1960), "Small Bowel Biopsy with the Suction Biopsy Tube", AUST. ANN. MED., in the press.

ROBERTSON, E. G., WOOD, I. J., and JOSKE, R. A. (1955), "Gastric Atrophy with Subsequent Pernicious Anæmia and Pronounced Subacute Combined Degeneration of the Cord", *Lancet*, **2**, 69.

ROSS, G. I. M. (1952), "Vitamin B_{12} Assay in Body Fluids Using *Euglena gracilis*", *J. clin. Path.*, **5**, 250.

SCHILLING, R. F. (1953), "Intrinsic Factor Studies. II. The Effect of Gastric Juice on the Urinary Excretion of Radioactivity after the Oral Administration of Radioactive Vitamin B_{12}", *J. Lab. clin. Med.*, **42**, 860.

SIURALA, M., and NYBERG, W. (1957), "Vitamin B_{12} Absorption in Atrophic Gastritis", *Acta med. scand.*, **157**, 435.

SPRAY, G. H., and WITTS, L. J. (1958), "Results of 3 Years' Experience with Microbiological Assay of Vitamin B_{12} in Serum", *Brit. med. J.*, **1**, 295.

WOOD, I. J., DOIG, R. K., MOTTERAM, R., and HUGHES, A. (1949*a*), "Gastric Biopsy: A Report of 55 Biopsies Using a New Flexible Gastric Biopsy Tube", *Lancet*, **1**, 18.

WOOD, I. J., DOIG, R. K., MOTTERAM, R., WEIDEN, S., and MOORE, A. (1949*b*), "The Relationship Between the Secretions of the Gastric Mucosa and its Morphology as Shown by Biopsy Specimens", *Gastroenterology*, **12**, 949.

WOOD, I. J., and TAFT, L. I. (1958), "Diffuse Lesions of the Stomach", Arnold, London.

BONE CHANGES ASSOCIATED WITH CORTISONE ADMINISTRATION IN THE RAT : CONVERSION OF " RICKETS " TO " OSTEOPOROSIS "[1]

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SUMMARY

Many studies of bone disorders showing density variation have been of a general metabolic type—intake, output and serum levels of calcium and other ions. These play an important part in the development of our knowledge, but, necessarily, give only an indication of average osseous change ; this is different in different bones. Morphological studies of several individual bones in any given case are thus an essential counterpart of the other form of inquiry.

Furthermore, it seems from the recent literature that osteoporosis and osteomalacia are coming be regarded clinically, more and more, as disease conditions or possibly syndromes, whereas they should not represent more than processes. To elucidate some aspects of these questions, experimental investgations have been undertaken.

Cortisone administration to rats with fully developed low-calcium/high-phosphorus " rickets ", characterized by widened osteoid seams and areas of bone resorption, results in disappearance of osteoid seams, diminution in size and number of bone trabeculæ and development of osteoporosis. Detailed studies of this change in bone structure show that in this form of rickets resorption is confined to calcified bone ; osteoid, lined by mast cells, does not resorb except where areas of sporadic calcification appear in it. With cortisone, osteoid material continues to calcify ; but new osteoid formation no longer appears, mast cells slowly disappear, and the area of calcified bone surface undergoing resorption increases in extent. Thus the anti-anabolic effect of cortisone, by inhibiting further matrix formation, is indirectly responsible for increased resorption. These results suggest that " rickets " and osteoporosis are interconvertible, and that the type of bone change, in the presence of abnormal calcium metabolism of any kind, may depend on the level of cortisone (or comparable hormones).

SINCE the work of Albright (1942–43) and Albright and Reifenstein (1948), osteoporosis has been defined as a condition resulting from failure of formation of bone matrix (Cooke, 1955 ; Snapper, 1957). There is, however, insufficient evidence to support this definition, including as it does a still disputed pathogenesis, and it is considered more appropriate to confine the definition to a purely descriptive one, such as the following : " Osteoporosis is a condition in which there is loss of bone substance associated with thinning and decrease in number of trabeculæ and honeycombing of compact bone."

The pathogenesis of the condition has been the subject of extensive clinical investigation since 1947, when Albright proposed an anti-anabolic theory to account for the diminution of bone mass in osteoporosis. Anti-anabolism was used to mean a diminution of bone mass resulting from a decrease in protein synthesis of matrix while normal bone resorption proceeded unchecked. This conception was supported by numerous clinical studies on man (Reifenstein and Albright, 1947 ; Reifenstein, 1958) and experimental studies on rabbits (Sissons and Hadfield, 1955). It is only recently that doubts have been raised regarding the validity of the " anti-anabolic " theory, and increased resorption of bone is now thought to play an important role in the development of osteoporosis (Storey, 1957, 1958*a*, 1958*b* ; Urist and Deutsch, 1960).

One problem in the study of osteoporosis is that, once bone is grossly porotic, histological examination gives little indication of the method of bone loss (Storey, 1957). A similar situation has been commented on by Follis (1951) in studies of osteoporosis associated with Cushing's syndrome, in which the remaining trabeculæ appeared of normal structure, with no evidence of excessive resorption or decreased formation of bone. Another histological problem lies in the recognition of areas of bone resorption. Although osteoclasts are frequently seen in areas of resorption, their absence does not necessarily mean that removal of bone is not

[1] Received on May 23, 1960.

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in progress. That resorption can occur in the absence of osteoclasts has been stated previously (Jaffe, 1930; King, 1935), and recent studies of experimental osteoporosis emphasize that vascular resorption is prominent and plays an important role in the process (Storey, 1957; Urist and Deutsch, 1960).

Many ætiological factors have been proposed to account for osteoporosis; but, in general, the literature has been dominated by the hormonal hypothesis of Albright (1947). This has been supported by observations that prolonged administration of cortisone and related adrenal cortical steroids induces osteoporosis (Sissons and Hadfield, 1955; Storey, 1957; Urist, 1958), and sex hormone treatment of patients with osteoporosis leads to calcium and nitrogen retention (Albright and Reifenstein, 1948). The significance of studies of this kind is difficult to assess, in that they indicate only the general direction of over-all change in the skeleton, and information appertaining to individual bones is not available. Although symptomatic improvement may occur, it has as yet been difficult to demonstrate radiographic or histological improvement (Meroney *et alii*, 1959). More detailed histological studies are clearly necessary.

An association between cortisone and calcium metabolism has recently been demonstrated which may help to throw fresh light on the problem of osteoporosis. It has been demonstrated that cortisone restores to normal the elevated serum calcium level of hypervitaminosis D and sarcoidosis, but not that of hyperparathyroidism (Anderson *et alii*, 1954; Verner *et alii*, 1958; Connor *et alii*, 1956). This effect led Anderson *et alii* (1954) to suggest that cortisone antagonized vitamin D and thus inhibited calcium absorption from the gut. Calcium deficiency usually leads to osteomalacia, which is characterized by decreased mineral content of bone and widened uncalcified osteoid seams, thus differing clearly from osteoporosis, although many patients with this condition are in negative calcium balance (Albright and Reifenstein, 1948; Urist, 1958). In the case of birds maintained on calcium-deficient diets, osteoporosis does develop instead of osteomalacia; but here hypertrophy of the adrenal cortex has been observed (Urist, 1959). Thus in calcium deficiency, whether it is primarily dietetic or secondary to elevated corticosteroid levels, osteoporosis can occur instead of osteomalacia; the determining factor may well be cortisone or related steroid, the concentration of which also determines the rate and degree to which either condition develops. That cortisone, in the presence of calcium-phosphorus imbalance, does determine the development of either experimental "rickets" or osteoporosis has already been demonstrated (Storey, 1960).

The present experiments were designed to test the effect of cortisone on fully developed rickets, in this case induced by a low-calcium, high-phosphorus rhachitic diet, in which both widened uncalcified osteoid seams and areas of resorption were prominent but the epiphyseal cartilage was narrower than normal (Shohl and Wolbach, 1936; Urist and McLean, 1957). After the administration of cortisone, bones rapidly became porotic, until only a shell of cortical bone remained. The early stages of this conversion from one bone change to another are described here in detail.

EXPERIMENTAL PROCEDURES

Thirty-two rats weighing 40 to 50 grammes, after one month on the Shohl "E" diet, were divided into two groups; one group was used as a control, and the other was given cortisone acetate (40 milligrammes per kilogram per day) by subcutaneous injection into the thigh. Rats were killed with ether at intervals in the preliminary period, and in pairs, control and cortisone-treated, at four, eight and 12 days after the administration of cortisone was begun.

The Shohl "E" diet is a variation of the Steenbock-Black diet deficient in vitamin D, with the phosphorus level increased with 3·85% KH_2PO_4 and the omission of $CaCO_3$; the calcium-phosphorus content of the diet is of the order of 0·05% to 1·0%.

The tibia and skull were removed, examined macroscopically, cut in longitudinal sections and fixed in equal parts of 10% formol-saline and absolute alcohol. Blocks of bone were embedded, after decalcification in 5% nitric acid, in paraffin wax or, undecalcified, in acrylic resin (Mills, 1957).

Sections were cut at 5μ to 7μ, stained with Ehrlich's hæmatoxylin and eosin, periodic-acid Schiff (PAS), Alcian blue, toluidine blue, Lillie's silver impregnation and von Kossa's silver stain. Radiomicrograms were taken with a Philips contact microradiograph on "Kodak" spectroscopic safety film (694–0).

RESULTS

PRELIMINARY PERIOD ON THE DIET (0 TO 30TH DAY)

Macroscopic Examination

After seven days, the epiphyseal cartilage of the tibia was slightly narrower and the metaphysis shorter than normal; by 14 days metaphyseal bone trabeculæ were diminished both in size and in number. The vault of the skull showed increased vascularity, particularly near sutures. By 21 days the metaphysis was thicker, white and opaque in contrast with the translucent grey-white normal metaphysis. After 30 days it consisted of a wide band of white opaque material, which cut with the consistency of hard cheese. Similarly, the cranial sutures were outlined by white opaque lines, and vessels were no longer visible.

Microscopic Examination

(1) Tibia. At 7 days, the epiphyseal cartilage was slightly narrower than normal, and metaphyseal bone trabeculæ were short and irregular. By 14 days, the epiphyseal cartilage was still narrower; in the metaphysis, trabeculæ were short and lined by osteoblasts. In some areas, particularly down the endosteal surface of the diaphysis, osteoid margins were beginning to appear. By 21 days, osteoid seams increased in extent and width throughout the bone. Numerous toluidine blue-staining mast cells now appeared at endosteal osteoid surfaces, particularly in the metaphysis. By 30 days, endochondral growth still progressed slowly, with cartilage remnants being incorporated into the metaphysis, which consisted largely of osteoid tissue partly encasing thin cores of calcified bone. In the osteoid tissue there were a few scattered areas opaque to X rays; osteoid matrix was lined by cuboidal to spindle-shaped osteoblasts and numerous mast cells; in areas where calcified bone was not covered with osteoid matrix, resorption was in progress.

The osteoid tissue failed to stain with hæmatoxylin or PAS, but did stain faintly with Alcian blue, whereas calcified bone stained with hæmatoxylin and PAS but not with Alcian blue. With von Kossa's stain in undecalcified sections, bone stained intensely and was radio-opaque, while osteoid tissue failed to stain and was radiolucent. Toluidine blue staining showed many mast cells associated with a capillary network (Figure III). The mast cell granules stained deeply with PAS and were metachromatic with toluidine blue; silver impregnation showed the osteoid matrix to be lamellar in structure, and revealed a fine network of reticular fibres in the intertrabecular spaces.

(2) Skull and Teeth. Changes similar to those in the long bones occurred in the skull, although here mast cells were not so numerous. Most trabecular margins were lined by a wide seam of osteoid tissue; this was prominent at the edges of sutures and around vessels. In a few areas calcification of osteoid material was in progress; these areas stained with silver, PAS and hæmatoxylin, and were radio-opaque (Figures IX and X). Scattered areas of resorption were also present.

In the teeth, a wide layer of predentine had formed, which failed to stain with PAS and hæmatoxylin except for large globules distributed irregularly in the predentine. Predentine was lined by odontoblasts, and mast cells were not present.

CONTROL PERIOD ON THE DIET (30TH TO 42ND DAY)

During this period, animals were killed at 4, 8 and 12 days, and the bones showed the same general changes as those described above, with the exception that osteoblasts became more spindle-shaped and areas of resorption were more noticeable (Figures I and V).

CORTISONE ADMINISTRATION (30TH TO 42ND DAY)

Macroscopic Examination

Four days after the administration of cortisone began, the metaphysis was reddish in colour, and by the end of 12 days only a small amount of bone remained connecting the diaphysis to the epiphyseal cartilage; in addition, the shafts of the long bones were extremely fragile. The cranial vault also showed increased vascularity.

Microscopic Examination

(1) Tibia. After four days, metaphyseal trabeculæ were reduced in number and size. The most significant change was the reduction in number of osteoblasts lining bone surfaces; those that remained were more spindle shaped, and instead of bone formation, resorption was beginning where extremely patchy areas of calcification in osteoid stained with fine granules of PAS or hæmatoxylin. Little change was apparent in mast cells.

By eight days, bones were becoming rarefied; only a few areas showed a patchy distribution of osteoid material, while trabecular margins were irregular owing to the appearance of large resorption cavities (Figure II). The most obvious cell was now the multinucleated osteoclast; these cells were present in great numbers and at different stages of development, usually lying in Howship's lacunæ. Osteoclasts containing cytoplasmic mucopolysaccharide granules were associated with removal of PAS and hæmatoxylin-staining calcified bone; but none adjoined osteoid seams. Mast cells were reduced in number, the majority remaining next to capillaries which were no longer adjacent to the osteoid surface.

After 12 days, bones were more rarefied; most of the metaphysis was replaced by vascular, cellular marrow, and trabecular surfaces were scalloped by numerous Howship's lacunæ in which osteoclasts were lying. Near or adjoining these areas were remnants of osteoid tissue, some calcifying and others, uncalcified, being undermined by bone removal. Although osteoblasts were less numerous than in the untreated animals, some were still present at bone surfaces



FIGURE I

Photomicrograph of metaphyseal area of tibia after 42 days on the Shohl " E " diet. The epiphyseal cartilage is narrower than in the rat on a normal diet, and the metaphysis consists largely of osteoid tissue encasing cartilage remnants or small areas of calcified bone. (Ehrlich's hæmatoxylin and eosin stain. ×120)



FIGURE II

Photomicrograph of metaphyseal area in the tibia of a rat on the Shohl " E " diet for 38 days and treated with cortisone from 30th to 38th day. The epiphyseal cartilage is narrowed, only remnants of osteoid remain, and extensive bone resorption is seen in the metaphysis. (Hæmatoxylin and eosin stain. ×120)

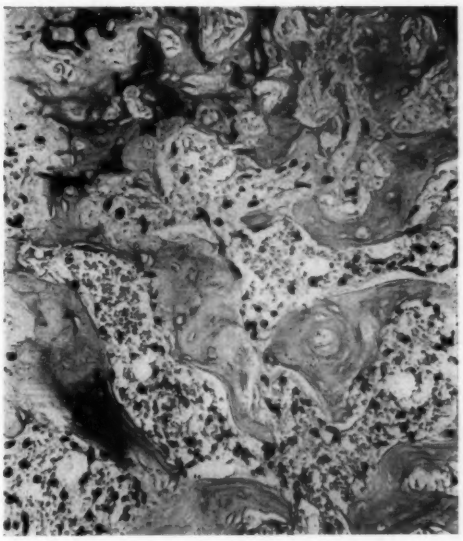


FIGURE III

Photomicrograph of metaphyseal trabeculæ in the tibia of a rat after 30 days on the Shohl " E " diet. Large numbers of deeply-staining mast cells are present near the bone margins. (Toluidine blue stain. ×120)

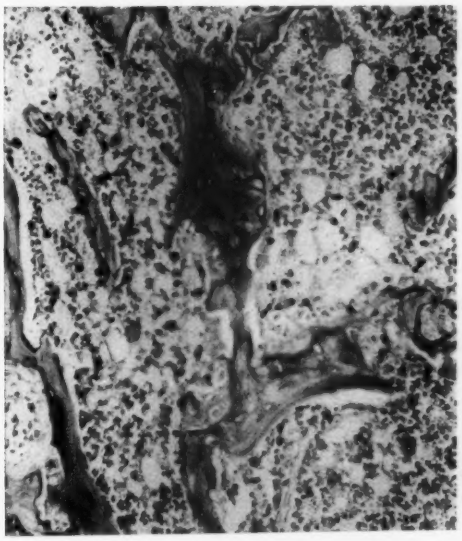


FIGURE IV

Photomicrograph of metaphyseal trabeculæ in the tibia of a rat after 42 days on the Shohl " E " diet and treated with cortisone from 30th to 42nd day. Deeply-staining mast cells are reduced in number compared with the control animals at either 30 or 42 days. (Toluidine blue stain. ×120)



FIGURE V

Photomicrograph of trabeculæ after 42 days on the Shohl " E " diet, showing lightly-staining wide osteoid seams. (Hæmatoxylin and eosin stain. ×120)



FIGURE VII

Photomicrograph of bone of cranial vault of a rat after 42 days on the Shohl " E " diet and treated with cortisone from the 30th to 42nd day, showing an area of resorption which has partly undermined an osteoid Haversian system. The periphery of this system now stains with hæmatoxylin, particularly where resorption is occurring. (Hæmatoxylin and eosin stain. ×180)



FIGURE VI

Photomicrograph of trabeculæ in a rat kept for 42 days on the Shohl " E " diet, treated with cortisone from the 30th to 42nd day, showing decreased size of trabeculæ, absence of osteoid seams and increased resorption of bone. (Hæmatoxylin and eosin stain. ×80)

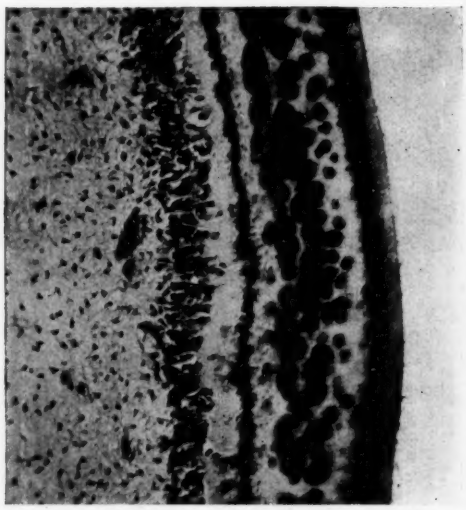


FIGURE VIII

Photomicrograph of upper incisor tooth of a rat after 42 days on the Shohl " E " diet and treated with cortisone from 30th to 42nd day, showing widened predentine, in which large globules characteristic of rickets are present. In the cortisone-treated animals a deeply-staining line is present near the odontoblastic layer. (Hæmatoxylin and eosin stain. ×120)



FIGURE IX

Photomicrograph of undecalcified section of the cranial vault of a rat after 30 days on the Shohl "E" diet, showing widened unstained osteoid seams with a small area of calcification shown by arrow. A small resorption cavity containing two osteoclasts is present at the margin of calcified bone on the lower right side of the section. (Von Kossa, hæmatoxylin and eosin stain. ×175)

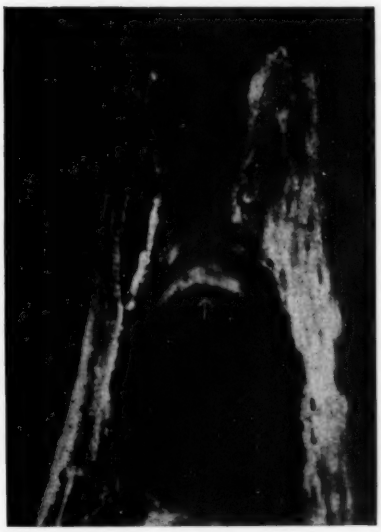


FIGURE X

Radiomicrogram of section shown in Figure IX. Areas staining with silver are radio-opaque; osteoid is radiolucent and contains an area (indicated by arrow) which has calcified. (×175)



FIGURE XI

Photomicrograph of undecalcified section of the cranial vault adjoining the sagittal suture in a rat after 42 days on the Shohl "E" diet and treated with cortisone from 30th to 42nd day. Osteoid is greatly reduced in extent, and numerous osteoclasts are present in Howship's lacunæ in calcified bone. (Hæmatoxylin and eosin stain. ×175)



FIGURE XII

Radiomicrogram of section shown in Figure XI. Areas staining deeply with hæmatoxylin are radio-opaque and osteoid is radiolucent. Large numbers of Howship's lacunæ are seen at calcified bone margins. (×175)

where there was bone formation. Mast cells, although reduced in number, were still present (Figures IV and VI).

(2) Skull and Teeth. Changes in the skull and teeth followed the same pattern; by the end of 12 days, the cranial vault was rarefied by resorption of calcified bone, and only a few areas of osteoid tissue remained intact; most had either disappeared or been undermined by continued resorption of calcified bone (Figures VII, XI and XII). In the teeth, a line staining with PAS and hæmatoxylin was now present in the widened predentine layer (Figure VIII).

DISCUSSION

Rats on a vitamin D deficient diet of low calcium and high phosphorus content fail to grow, and develop a form of rickets characterized by slight narrowing of epiphyseal cartilage and the development of a dense, but largely uncalcified, metaphysis. This deviation from the normal growth pattern is brought about by a change in the balance of the two processes, deposition and resorption, responsible for remodelling of bone. Although epiphyseal cartilage is narrow, more bone matrix is formed than is removed by resorption, so that the metaphysis, largely uncalcified, consists mainly of osteoid tissue. When calcified bone is not encased by osteoid matrix, it is being resorbed. Failure of removal of uncalcified bone matrix may be due to the lack of calcification, for osteoid tissue appears not to resorb until it calcifies (Bailie and Irving, 1955). This has also been demonstrated during parathormone administration in rhachitic rats, in which calcified bone is resorbed instead of uncalcified matrix (Weinmann and Sicher, 1955). Similarly, in sporadically laying hens receiving a diet of low calcium content, osteoclasts appear almost exclusively next to calcified bone and are absent from osteoid margins (Bloom *et alii*, 1958). Recent observations have shown that in naturally occurring renal osteodystrophy in man, both widened osteoid seams and increased resorption spaces can occur in the same bone trabeculæ. Combined microradiographic and histological study supports the hypothesis that only calcified bone is resorbed. The formation of extensive osteoid tissue on bone margins leaves relatively few calcified bone surfaces exposed to surrounding tissue cells, and hence reduces the area available for bone resorption. One consequence of this may be the extremely low serum calcium levels recorded in low-calcium, high-phosphorus rickets (Urist and McLean, 1957), despite hypertrophy of the parathyroid gland (Carnes *et alii*, 1942).

During the administration of cortisone, osteoid formation slackens, but calcification of existing uncalcified matrix continues, and concomitantly resorption is increased. With decreased deposition of uncalcified matrix, the surface area involved in the resorptive process increases, so that bones become increasingly rarefied. The stimulus for resorption is probably the low serum calcium levels found in this type of rickets due to the low dietary level which is known to be associated with hypertrophy of the parathyroid gland (Carnes *et alii*, 1942). Whether cortisone induces an added stimulus for bone resorption, or merely permits resorption to continue unchecked by further osteoid formation, is not known.

Calcification occurs sporadically in low-calcium, high-phosphorus rickets (Urist and McLean, 1957), and is well demonstrated by large characteristic globules in the widened predentine of the continuously growing incisor tooth. Such globules have been described previously in rickets by Becks and Ryder (1931). They have the staining properties of mucopolysaccharides; similar areas in osteoid tissue stain similarly and are radio-opaque. This supports the suggestion that mucopolysaccharides are intimately associated with the calcification process (Sobel, 1954) and, particularly in the present circumstances when there is a low level of calcium in the diet, act as a booster mechanism. The question as to whether the increased rate of resorption of bone with liberation of calcium, or disturbed dentine matrix formation, is responsible for the line of calcification in the predentine occurring during cortisone treatment, still remains to be answered.

The presence of mast cells at endosteal bone margins in this form of rickets is not understood (Urist and McLean, 1957). With cortisone they slowly decrease in number, and those that remain appear farther away from the bone; however, they still remain associated with small capillaries, and may be concerned here with maintenance of capillary integrity in the presence of rapid remodelling of bone and extremely low levels of ionized calcium.

Rickets does not always result from diets apparently optimal for its development, particularly if animals fail to grow (Park, 1954). In low-calcium as well as high-calcium rickets, starvation with resultant loss of weight is associated with calcification and healing of rhachitic epiphyseal cartilage (Thomas *et alii*, 1957). As adrenal hypertrophy occurs during starvation (Mulinos and Pomerantz, 1940), this, together with the present observations, suggests that hormones of the adrenal cortex

may play a modifying role in the development of rhachitic changes, and, in certain conditions, modulations between "osteomalacia" and "osteoporosis" are likely. During such transformations, the time at which bone is studied and the relative rates of the two component processes—resorption and osteoid formation—will determine the histological picture. Such mixed pictures have, indeed, been recorded in renal osteodystrophy, in which changes range from widened osteoid seams to areas of rapid bone resorption, with combinations of the two occurring in the same bone trabeculæ (Follis and Jackson, 1943; Stanbury, 1957).

ACKNOWLEDGEMENTS

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REFERENCES

ALBRIGHT, F. (1942–43), "Cushing's Syndrome: Its Pathological Physiology: Its Relationship to the Adreno-Genital Syndrome and Its Connection with the Problem of the Reaction of the Body to Injurious Agents ('Alarm Reaction' of Selye)", *Harvey Lect.*, **38**, 123.

ALBRIGHT, F. (1947), "Osteoporosis", *Ann. intern. Med.*, **27**, 861.

ALBRIGHT, F., and REIFENSTEIN, E. C., JR. (1948), "The Parathyroid Glands and Metabolic Bone Disease. Selected Studies", Williams & Wilkins, Baltimore.

ANDERSON, J., DENT, C. E., HARPER, C., and PHILPOT, G. R. (1954), "Effect of Cortisone on Calcium Metabolism in Sarcoidosis with Hypercalcæmia—Possibly Antagonistic Actions of Cortisone and Vitamin D", *Lancet*, **2**, 720.

BAILIE, J. M., and IRVING, J. T. (1955), "Development and Healing of Rickets in Intramembranous Bone", *Acta med. scan.*, **152**, Supp. 306, 1.

BECKS, H., and RYDER, W. B. (1931), "Experimental Rickets and Calcification of Dentin", *A.M.A. Arch. Path.*, **12**, 358.

BLOOM, M. A., DOMM, L. V., NALBANDOV, A. V., and BLOOM, W. (1958), "Medullary Bone of Laying Chickens", *Amer. J. Anat.*, **102**, 411.

CARNES, W. H., PAPPENHEIMER, A. M., and STOERK, H. C. (1942), "Volume of Parathyroid Glands in Relation to Dietary Calcium and Phosphorus", *Proc. Soc. exp. Biol. (N.Y.)*, **51**, 314.

CONNOR, T. B., HOPKINS, T. R., THOMAS, W. C., JR, CAREY, R. A., and HOWARD, J. R. (1956), "The Use of Cortisone and A.C.T.H. in Hypercalcæmic States", *J. clin. Endocr.*, **16**, 945.

COOKE, A. M. (1955), "Osteoporosis", *Lancet*, **1**, 929.

FOLLIS, R. H., JR (1951), "The Pathology of the Osseous Changes in Cushing's Syndrome in an Infant and in Adults", *Bull. Johns Hopk. Hosp.*, **88**, 440.

FOLLIS, R. H., JR, and JACKSON, D. A. (1943), "Renal Osteomalacia and Osteitis Fibrosa in Adults", *Bull. Johns Hopk. Hosp.*, **72**, 232.

JAFFE, H. L. (1930), "The Resorption of Bone. A Consideration of the Underlying Processes Particularly in Pathologic Conditions", *A.M.A. Arch. Surg.*, **20**, 355.

KING, E. S. J. (1935), "Localized Rarefying Conditions of Bone", Arnold, London.

MERONEY, W. H., RUBINI, M. E., ROSCH, P. J., AUSTEN, F. K., HERNDON, E. G., JR, and BLYTHE, W. B. (1959), "Decreased Density of Bone: An Etiologic Approach to Diagnosis", *Metabolism*, **8**, 293.

MILLS, K. W. (1957), "Section-Cutting of Un-Decalcified Bone", *Aust. N.Z. J. Surg.*, **26**, 262.

MULINOS, M. G., and POMERANTZ, L. (1940), "Pseudo-Hypophysectomy. A Condition Resembling Hypophysectomy Produced by Malnutrition", *J. Nutr.*, **19**, 493.

PARK, E. A. (1954), "The Influence of Severe Illness on Rickets", *Arch. Dis. Child.*, **29**, 369.

REIFENSTEIN, E. C., JR (1958), "Control of Corticoid-Induced Protein Depletion and Osteoporosis by Anabolic Steroid Therapy", *Metabolism*, **7**, 78.

REIFENSTEIN, E. C., JR, and ALBRIGHT, F. (1947), "The Metabolic Effects of Steroid Hormones in Osteoporosis", *J. clin. Invest.*, **26**, 24.

SHOHL, A. T., and WOLBACH, S. B. (1936), "Rickets in Rats. XV. The Effect of Low Calcium-High Phosphorus Diets at Various Levels and Ratios upon the Production of Rickets and Tetany", *J. Nutr.*, **11**, 275.

SISSONS, H. A., and HADFIELD, G. J. (1955), "The Influence of Cortisone on the Structure and Growth of Bone", *J. Anat. (Lond.)*, **89**, 69.

SNAPPER, I. (1957), "Bone Diseases in Medical Practice", Grune & Stratton, New York.

SOBEL, A. E. (1954), "Local Factors in the Mechanism of Calcification", *Ann. N.Y. Acad. Sci.*, **60**, 713.

STANBURY, S. W. (1957), "Azotæmic Renal Osteodystrophy", *Brit. med. Bull.*, **13**, 57.

STOREY, E. (1957), "The Effect of Continuous Administration of Cortisone and Its Withdrawal on Bone", *Aust. N.Z. J. Surg.*, **27**, 19.

STOREY, E. (1958*a*), "The Effect of Intermittent Cortisone Administration in the Rabbit", *J. Bone Jt Surg.*, B, **40**, 103.

STOREY, E. (1958*b*), "The Influence of Cortisone and A.C.T.H. on Bone Subjected to Mechanical Stress (Tooth Movement)", *J. Bone Jt Surg.*, B, **40**, 558.

STOREY, E. (1960), "Bone Changes Associated with Cortisone Administration in the Rat: Effect of Variations in Dietary Calcium and Phosphorus", *Brit. J. exp. Path.*, **41**, 207.

THOMAS, W. C., HOWARD, J. E., and CONNOR, T. B. (1957), "Studies on Rickets Induced by a Low Calcium Diet, Effect of Starvation, Citrates and Succinates", *Bull. Johns Hopk. Hosp.*, **101**, 123.

URIST, M. R. (1958), "The Problem of Osteoporosis", *Clin. Res. Proc.*, **6**, 377.

URIST, M. R. (1959), "The Effects of Calcium Deprivation upon the Blood, Adrenal Cortex, Ovary, and Skeleton in Domestic Fowl", *Advanc. Hormone Res.*, **15**, 455.

URIST, M. R., and DEUTSCH, N. M. (1960), "Osteoporosis in the Laying Hen", *Endocrinology*, **66**, 377.

URIST, M. R., and MCLEAN, F. C. (1957), "Accumulation of Mast Cells in Endosteum of Bones of Calcium Deficient Rats", *A.M.A. Arch. Path.*, **63**, 239.

VERNER, J. V., JR, ENGEL, F. L., and MCPHERSON, H. T. (1958), "Vitamin D Intoxication: Report of Two Cases Treated with Cortisone", *Ann. intern. Med.*, **48**, 765.

WEINMANN, J. P., and SICHER, H. (1955), "Bone and Bones. Fundamentals of Bone Biology", 2nd Edition, Mosby, St. Louis.

A Review Written by Request

CALCIUM METABOLISM

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CALCIUM METABOLISM

DISORDERS of calcium and phosphorus metabolism are by no means uncommon. They may be found in association with diseases of the bones, the kidneys and the gastro-intestinal tract, and whatever their origin, they may result in involvement of many organs and tissues of the body, producing diverse clinical manifestations.

Before proceeding to more clinical aspects of the subject, it is necessary to deal with some general physiological considerations. The three main sites of regulation of calcium and phosphorus metabolism, as at present understood, are the intestine, which is the portal of entry, the bones, which constitute the storehouse, and the kidneys, which provide the portal of exit. The possible role of the intestine as an additional excretory channel is debated. There are two chief physiological regulating devices—namely, the parathyroid secretions and vitamin D.

Calcium Balance and Exchange

The daily intake of calcium is commonly about 1000 mg., but it varies a great deal, depending particularly on the ingestion of milk and milk products, which supply much of the dietary calcium. Egg yolk and some fish foods are other rich sources. Whatever the total intake, the daily net absorption of calcium is relatively small, being about 100 to 250 mg. There are a few individuals in whom intestinal absorption is greatly enhanced by the ingestion of large amounts of calcium.

Since normal adults are in calcium balance, the daily urinary excretion of calcium is equal to the daily net absorption of about 100 to 250 mg. In certain physiological conditions (for example, pregnancy and lactation), calcium is lost from the body by other channels, and balance is modified accordingly. This loss may be temporarily uncompensated, as in the production of tetany in domestic cows (the syndrome known as " milk fever "). Hodgkinson and Pyrah (1958) have recently emphasized that the urinary calcium excretion is less than 300 mg. per day in over 90% of normal human subjects taking ordinary diets. Furthermore, Knapp (1947) has shown that the apparent variation in urinary excretion of calcium from individual to individual may be reduced considerably by making allowance for body weight. It follows that the determination of 24-hour urinary calcium excretion is a useful test in the investigation of patients who are thought to be suffering from disorders of calcium metabolism and should be performed more often, without the necessity for a preliminary period of equilibration on a diet of known calcium content.

The amount of calcium present in non-osseous tissues of normal adults is about 10 grammes, and in comparison with this it is important to note that more than 1000 grammes (99%) are present in the bones. This fact is of cardinal importance in the investigation of disorders of calcium metabolism, for we can determine the calcium balance with considerable accuracy by measuring the dietary and stool calcium contents, in addition to urinary calcium excretion, over a period of some days or weeks. It is apparent that any change in calcium balance during such a period will be a measure of changes occurring in skeletal stores.

Bone Mineralization

Investigation of the chemical and histological structure of bone has shown that it consists of an organic matrix, which contains collagen, and a mucoprotein (the osteoid), upon which

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calcium is laid down as complex calcium phosphates (known as apatites). Hydroxyapatite, which is the commonly occurring form, has a basic formula corresponding to $Ca_{10}(PO_4)_6(OH)_2$, which may be modified by internal substitutions and defects within the molecule, and which is also subject to surface substitutions particularly with sodium, magnesium, carbonate and citrate. These reactions account for the variable composition of bone mineral. Although there is as yet no uniformity of opinion, a good deal is known about the molecular configuration and spatial orientation of apatite, and this orientation depends in part on the properties of the organic matrix. It appears that apatite is laid down as minute crystals of the order of 200 by 30–70 Ångström units in size, and with the crystalline *c*-axis parallel to the longitudinal direction of the collagen fibres. Such crystals present an enormous surface area for exchange of calcium and phosphorus between bone and extracellular fluids.

It is an old observation that bone is a dynamic tissue, in which continuous deposition and resorption are associated with a large daily exchange of calcium and phosphorus between bone and bloodstream. More recently, the use of radioactive isotopes has provided further understanding of the process. It has been shown that the exchange occurs particularly in the newly-formed superficial layers of bone. The older, deeper layers are relatively inactive, though they may become involved where new bone formation is occurring. This explains in part the inaccessibility of radioisotopes deposited in the skeleton and the failure of therapeutic measures to remove them.

Studies of physical chemistry have produced some new concepts of the mechanisms of dynamic exchange between bone and blood-stream (Neuman and Neuman, 1958). It has been shown that plasma is normally *supersaturated* with calcium and phosphorus in relation to bone mineral, so that the purely physicochemical tendency is for mineral deposition. Furthermore, crystallographic studies increasingly emphasize the importance in mineral deposition of seeding from the supersaturated plasma of the organic matrix with apatite crystals. It follows that there must be an active mechanism under physiological control to facilitate bone mineral dissolution and the transport of calcium and phosphorus from bone to blood-stream. These facts have necessitated some reappraisal, not only of the chemical processes of bone deposition and resorption, but also of the actions of the physiological regulators (parathyroid secretions, vitamin D and alkaline phosphatase).

The Plasma Calcium

The plasma calcium exists as three separate fractions which are in equilibrium. Recent work has shown that 60% to 70% of the total plasma calcium is ultrafiltrable, and most of the ultrafiltrable portion is ionized calcium (Rose, 1957; Fanconi and Rose, 1958). The small unionized ultrafiltrable fraction consists chiefly of calcium complexes with organic acids, inorganic phosphates and carbonates. The nonultrafiltrable portion is protein-bound, chiefly to the plasma albumin, and is unionized.

Despite the large daily turnover of calcium between bone and blood-stream and from intestine to blood-stream to kidney, the total plasma calcium is normally maintained within the relatively narrow range of about 9 to 11 mg. per 100 ml. Furthermore, quite small deviations of the plasma calcium from its normal range may produce profound symptoms and extensive disease. Such fine adjustment in health results particularly from the physiological regulation exercised by the parathyroids and vitamin D.

Phosphorus Metabolism

Changes in calcium metabolism are usually associated with changes in phosphorus metabolism. Like calcium, phosphorus is absorbed from the intestine (as inorganic phosphate), excreted by the kidney and stored in bone. Nevertheless, there are several important points of difference between the two. First, although the phosphorus of plasma inorganic phosphate (referred to subsequently as plasma phosphorus) is normally maintained within a range of about 3 to 5 mg. per 100 ml. in adults, it may vary considerably from 0·5 to 15 mg. per 100 ml. without of itself producing symptoms. Secondly, the problem of limited absorption, which is so important in calcium metabolism, does not apply to phosphorus, which is freely absorbed from the gut, and of which any excess is excreted in the urine. The kidney has a fairly sharp threshold for phosphorus, and the 24-hour excretion in the urine varies considerably in health, reflecting particularly the dietary intake. Thirdly, only about half of the total phosphorus content of the body is found in the bones, the remainder comprising the important organic phosphate compounds which are found throughout the tissues and fluids of the body. These differences between calcium and phosphorus metabolism have been well shown diagrammatically by Dent (1956).

Physiological Regulation

The Parathyroids.—The chief function of the parathyroid glands is to regulate the mobilization

of calcium from bone to blood-stream, so as to maintain a normal plasma calcium level. The plasma calcium levels determine parathyroid activity, and hypocalcæmia is the best known stimulus to parathyroid secretion. It is improbable that an elevated plasma phosphorus level is of itself an adequate stimulus for such secretion, and there is no convincing evidence of a pituitary parathyrotrophic principle.

It is well known that the parathyroid secretions tend to raise the plasma calcium level, to lower the plasma phosphorus level, and to produce an increased urinary excretion of both calcium and phosphorus. Furthermore, it is generally agreed that they have an action on the kidneys and an action on the bones; but there has been much disagreement about the nature and relation of these actions one to another.

Some of the early investigators (Collip *et alii*, 1925) argued that the prime effect was to liberate calcium and phosphorus from bone to blood-stream, the ensuing changes in circulating electrolytes being secondary. Such a theory alone could not explain the low plasma phosphorus level which is a common feature of hyperparathyroidism in the absence of complicating renal insufficiency.

In 1929, Albright and Ellsworth showed that the administration of parathyroid extracts to hypoparathyroid individuals was followed by an immediate increase in urinary phosphorus excretion and a fall in plasma phosphorus level. The plasma calcium level soon rose, but the increased excretion of calcium in the urine was delayed for some hours. By 1934, Albright and his co-workers had recognized that hyperparathyroidism, which was originally described as a disease of bone, could also occur in the absence of such changes. Indeed, these cases, which usually present with renal manifestations, are more common than the others. From these observations, Albright (Albright and Reifenstein, 1948) developed his concept that the prime action of the parathyroid hormone was on the kidney, producing the classical metabolic changes in the following way. (i) The parathyroid hormone lowered the renal threshold for phosphorus, increasing the urinary excretion and lowering the plasma level of phosphorus. (ii) The plasma calcium level rose as the phosphorus level fell, in order to maintain the constancy of some calcium-phosphorus solubility product. (iii) The increasing plasma calcium level then led to further loss of calcium in the urine. (iv) Unless this urinary loss was compensated by increased intestinal absorption, the hyperparathyroid subject passed into negative calcium balance, with depletion of skeletal stores and bony changes.

This hypothesis, which regarded bony changes as a secondary phenomenon, provided a ready explanation of the low plasma phosphorus level and of the occurrence of hyperparathyroidism with and without bone disease; but eventually it was challenged on many grounds. In particular, Barnicot (1948) produced direct local dissolution of bone by transplanting parathyroid tissue into its close proximity, and several groups showed that parathyroid extracts could act on bone and raise the plasma calcium level in the absence of the kidneys. It is not possible to review all this work, and it will suffice to say that most workers now accept the fact that the parathyroid secretions have direct and independent actions on both bone and kidney, mobilizing calcium and phosphorus from bone to blood-stream and phosphorus from blood-stream to urine. Teleologically, this is an admirable concept if the principal function of the parathyroids is to maintain a normal plasma calcium level by stimulating the transfer of calcium from bone. The phosphorus diuretic action is then concerned with ridding the blood-stream of the phosphorus necessarily liberated with the calcium (Dent, 1953).

There is some evidence, derived largely from studies of clinical material, that the parathyroid glands may produce two different hormones with these different actions. It is unfortunate that we still await the isolation from crude parathyroid extracts of a really pure chemical or chemicals with appropriate physiological activity, and this is the reason for much persisting uncertainty about fundamental problems of parathyroid physiology.

The precise mode of action of the parathyroid secretions on bone has been the subject of intensive investigation. Some evidence suggests that parathormone may promote resorption of the organic matrix with simultaneous dissolution of apatite, and that proteolytic or depolymerizing enzymes of bone matrix may be the final agents in producing demineralization under the influence of the parathyroid secretions.

On the other hand, Neuman and Neuman (1958) postulate that the parathyroid hormone increases the solubility of bone mineral by modifying the intermediary metabolism of bone cells so as to produce an increased citrate concentration. If the plasma is normally supersaturated with calcium and phosphorus in relation to bone mineral, there must be an active mechanism to ensure the transport of calcium and phosphorus from bone to blood-stream so as to maintain the dynamic equilibrium of deposition and resorption. This transport

is presumably regulated by the parathyroids, and citrate may play a vital role in the process. Bone is rich in citrate (Dickens, 1941), which is an admirable substance for the purpose, for, among other desirable properties, it forms with calcium a chelate in which calcium is soluble but unionized and may therefore be transported against an ionic gradient. Neuman and his co-workers (1956) have demonstrated a considerable increase in citrate output from bone almost immediately after the injection of parathyroid extracts, and they summarize their conclusions as follows: "... a parathyroid-controlled citrate gradient maintains a steady but supersaturated level of ionized calcium in serum."

Vitamin D.—The best known and probably the most important action of vitamin D is to promote the intestinal absorption of calcium, and this plays a vital role in the maintenance of normal plasma calcium levels. Calcium absorption will not proceed adequately in its absence, at any rate in the human. In vitamin D deficiency low plasma calcium and phosphorus levels are the characteristic chemical findings; skeletal changes (rickets and osteomalacia) may follow. The ready response of all these features to the administration of vitamin D can be explained as a consequence of the intestinal absorptive action, the lack of which is of prime importance in the production of rickets.

Yet the possibility exists of a direct physiological action of the vitamin on bone. There is no doubt that in very large doses vitamin D has a direct decalcifying effect on bone, though there is division of opinion that this is of importance in more physiological circumstances. Neuman and Neuman (1958) interpret available evidence to favour a normal direct decalcifying action which facilitates the effect of the parathyroid secretions, so that the two great physiological regulators of calcium metabolism work synergistically in controlling bone mineral dissolution.

The renal actions of vitamin D require further clarification. In hypoparathyroidism, Albright and his co-workers (Albright and Reifenstein, 1948) showed that it stimulated phosphorus excretion; but it has produced phosphorus retention in other circumstances. Recently, Litvak *et alii* (1958) have described the production of a low renal threshold for calcium. Actions of vitamin D on other tissues have also been described.

Alkaline Phosphatase.—Alkaline phosphatase is an enzyme which has the property of hydrolysing organic phosphate esters with the production of inorganic phosphate. It is produced by bone cells, and is found in relatively high concentration wherever calcification is occurring. A small amount is normally present in serum, and the serum alkaline phosphatase level is raised in most conditions in which there is excessive osteoblastic activity.

Increasing understanding of the physical chemistry of mineralization has failed to support the hypothesis, accepted for many years, that the role of alkaline phosphatase in bone metabolism was to facilitate mineral deposition by increasing the local concentration of inorganic phosphate and providing a "booster" mechanism. It has been shown on physical chemical grounds (Neuman and Neuman, 1958) that such a phosphate booster alone would be unable to achieve mineralization, and recent work on crystal seeding provides an alternative mechanism. Alkaline phosphatase may play a subsidiary part in mineralization, or it may have some other role, the nature of which is an interesting speculation. An attractive theory is that it is primarily concerned with matrix formation rather than mineralization of matrix, for this might explain why it is also found in tissues other than bone and cartilage.

SOME CLINICAL ASPECTS

Disorders of calcium and phosphorus metabolism may present in many ways, and patients are found in medical, surgical, pædiatric, orthopædic, urological, neuro-surgical, ophthalmological, dermatological and psychiatric clinics. It is not possible to review the diverse symptomatology of all these disorders, and attention will be directed to alterations of the plasma calcium level. Whatever the manifestations which bring the patient under notice, one must always be on the lookout for hypercalcæmia and hypocalcæmia, which produce rather characteristic clinical syndromes and may of themselves cause extensive disease.

Hypocalcæmia

It is well known that a low plasma calcium level may cause increased excitability of the peripheral nervous system, as manifested by numbness, tingling, cramps and spasms, particularly involving the hands and feet. This symptom complex, which is known as tetany, was brilliantly described by Trousseau. Other hypocalcæmic manifestations arise in or result from involvement of the central nervous system. Mental symptoms of some degree are probably universal, epilepsy and electroencephalographic abnormalities are not uncommon, while raised intracranial tension with papillœdema and also calcification of the basal ganglia are seen, particularly in hypoparathyroidism (Sugar, 1953; Grant, 1953).

Dermatological manifestations include a coarse, dry and scaly skin which may be associated with erythematous eruptions, dry, brittle hair which is easily shed, and brittle nails (Dent and Garretts, 1960). Laryngeal spasm with stridor (" laryngismus stridulus ") is not uncommon, particularly in childhood, and ocular cataracts are well known. Very rarely headache, abdominal pain and asthma have been described as hypocalcæmic manifestations.

The causes of hypocalcæmia are shown in the following tabulation

1. Deficiency of parathyroid secretions. Hypoparathyroidism :
   (*a*) Surgically induced.
   (*b*) Idiopathic.
   (*c*) Pseudohypoparathyroidism.
2. Deficiency of vitamin D (often associated with rickets or osteomalacia) :
   (*a*) Simple dietary deficiency (classical rickets, now rare).
   (*b*) Intestinal malabsorption syndromes.
3. Resistance to vitamin D and/or renal-tubular defects (the total calcium content is often normal in all these conditions) :
   (*a*) Vitamin D-" resistant " rickets.
   (*b*) The Fanconi syndrome.
   (*c*) Renal-tubular acidosis.
4. Renal-glomerular failure.
5. Miscellaneous :
   (*a*) Low plasma protein level.
   (*b*) Acute pancreatitis.

and the differentiation of these conditions usually presents little difficulty. A high plasma phosphorus level and a normal blood urea level are characteristic findings of hypoparathyroidism, while the phosphate retention of renal-glomerular failure is associated with urea retention and other evidence of poor renal function. In vitamin D deficiency and renal-tubular lesions, the plasma phosphorus level is low and the serum alkaline phosphatase level is raised, while family history, dietary history, other features of malabsorption and the presence or absence of glycosuria, amino-aciduria or systemic acidosis will usually serve to distinguish between the various members of these groups. Renal-tubular syndromes may be complicated by renal-glomerular failure. The urinary excretion of calcium is usually diminished in hypocalcæmia, although it may be normal or increased in the Fanconi syndrome and renal-tubular acidosis.

When the plasma protein level is low, the total plasma calcium content is often decreased ; but this is not a primary disorder of calcium metabolism, and the ionized calcium level, which is more important physiologically, is usually normal in these cases. The mechanisms of the production of a low plasma calcium level in acute pancreatitis are largely unknown.

Hypercalcæmia

Many of the symptoms of a raised plasma calcium level are related to loss of muscle tone. Generalized muscular weakness is common, and muscular hypotonicity may be demonstrable. Constipation is frequent ; difficulty may be experienced with ocular accommodation. Anorexia, nausea and sometimes vomiting may occur. Mental symptoms are not unusual. When the plasma calcium level is very high, cardiac standstill and sudden death may follow.

Renal manifestations are important. Polyuria is common, and is often associated with dryness of the mouth and thirst. Unless renal function is impaired, a raised plasma calcium level usually produces increased urinary calcium excretion ; but since it may also produce calcium deposition in many tissues including the kidneys, the features of renal-glomerular failure often complicate those of hypercalcæmia, whatever its cause.

Calcium deposition may occur in the ocular tissues, particularly as thin linear deposits of coarsely granular (" gritty ") appearance in the cornea close to the corneo-scleral junction on medial and lateral sides. These deposits must be distinguished from the more common " arcus senilis ", which extends more widely around the cornea and is less granular in appearance. Though by no means an invariable finding of hypercalcæmia, this characteristic corneal calcification when present is an important physical sign that the plasma calcium level is, or has been, raised.

The causes of hypercalcæmia are shown in the following tabulation.

1. Vitamin D overdosage :
   (*a*) From self-medication with proprietary medicines and vitamins.
   (*b*) Iatrogenic.
2. Sarcoidosis (with over-sensitivity to small doses of vitamin D).
3. Milk-alkali syndromes.
4. " Idiopathic " hypercalcæmia of infancy.
5. Hyperparathyroidism.
6. Osteoporosis — Some forms, particularly (*a*) immobilization, especially in childhood, (*b*) thyrotoxicosis, (*c*) Cushing's syndrome.
7. Paget's disease of bone (in periods of increased activity).
8. Secondary carcinoma involving bone.
9. Multiple myeloma.
10. Leukæmia, Hodgkin's disease and other reticuloses involving bone.
11. Carcinoma, sarcoma and reticuloses without bony involvement (rarely).
12. Adrenal insufficiency :
   (*a*) Addison's disease (rarely).
   (*b*) Acute, following adrenalectomy.

A high plasma calcium level may present as an incidental finding after bilateral adrenalectomy, in Paget's disease of bone, in secondary

carcinoma, in myeloma and in the reticuloses, when the diagnosis is well established and its cause is apparent. More often the diagnosis is not clear, and it is generally more difficult to differentiate the causes of hypercalcæmia than of hypocalcæmia. The presence or absence of hyperparathyroidism often demands careful consideration. The plasma phosphorus level is characteristically low in hyperparathyroidism, while it is normal or raised in the other conditions; but renal involvement is frequent, and the phosphorus level may be normal or raised in hyperparathyroidism with complicating renal-glomerular failure. Furthermore, a low plasma phosphorus level has been reported in almost all the conditions listed in the table (Thomas *et alii*, 1959), so that its diagnostic value is limited.

The serum alkaline phosphatase level is raised when hyperparathyroidism is complicated by bone disease, and in these cases specific radiological findings may make diagnosis relatively simple, though differentiation from "secondary hyperparathyroidism" can be most difficult if renal failure is also present. Numerous tests have been devised in an effort to facilitate the diagnosis of hyperparathyroidism (Thomas *et alii*, 1958), for no single procedure yet available will provide the answer in all cases. The plasma calcium level responds reciprocally when the plasma phosphorus level is lowered and raised in hyperparathyroid subjects, and this phenomenon forms the basis of a useful test (Chambers *et alii*, 1956). Administration of cortisone will correct the hypercalcæmia of sarcoidosis and many other conditions, but is without effect in hyperparathyroidism (Dent, 1956). Direct determination of the ionized calcium and other plasma fractions is often useful (Lloyd and Rose, 1958). Various procedures are used to measure the phosphorus diuretic action, but none is completely satisfactory or specific (Thomas *et alii*, 1959).

Additional Physical Findings

In every case a careful history and a complete physical examination are essential. Certain specific findings should be sought during the physical examination. Ocular calcification and tetany have been mentioned. Latent tetany may sometimes be made manifest by the application of a sphygmomanometer cuff to the extremities (Trousseau's sign).

The measurement of height and body proportions is too often omitted, for these simple measures may be of great value in assessing the site, degree and type of bone disease. In most normal individuals the span is equal to the height, while the two components of total height—crown-pubis and pubis-heel—are equal one to another. In senile osteoporosis the crown-pubis distance is usually several inches less than that from pubis to heel, though the latter is about half the span, while in osteoporosis due to hypogonadism the same general relations apply, but the span is considerably longer than is normal for the population and is often much in excess of 72 in. Conversely, in rickets the crown-pubis distance frequently exceeds that from pubis to heel and may be more than half the span.

Urinary examination should include the reagent which Sulkowitch developed to provide a rough bedside test of the concentration of calcium in the urine. It consists of a dilute solution of oxalic acid and ammonium oxalate with acetic acid in water, and to perform the test about equal quantities of the reagent and urine are mixed in a clean test tube (Albright and Reifenstein, 1948). When calcium is present in the urine, a fine precipitate of calcium oxalate forms, the speed of appearance and the density of the dispersed precipitate providing an indication of the amount of calcium present. Interpretation requires an assessment of the concentration of the urine, for a patient with hypercalciuria and polyuria may excrete 600 mg. in four litres of urine which has a lower concentration of calcium that that of a normal individual excreting 200 mg. in a volume of one litre. However, these two specimens of urine will be quite different in appearance, and with a little experience the test does provide a useful indication that the urinary calcium excretion is normal, increased or decreased.

LABORATORY DIAGNOSIS

Whatever the conclusions from clinical examination, final diagnosis requires a good laboratory service. Initial assessment can be made from determinations of plasma (or serum) calcium and phosphorus levels, serum alkaline phosphatase level, blood urea content and plasma protein and electrolyte content, together with the 24-hour urinary excretion of calcium and phosphorus, while radiology may provide important information about the bones and the kidneys. Final assessment often requires much other investigation.

It is proposed in conclusion to make some comments about the laboratory determination of calcium. All available methods require meticulous care to produce consistent results, yet an accuracy of about $\pm 1\%$ is desirable. Such precision makes heavy demands on a routine biochemical laboratory, and metabolic laboratories sometimes fail to confirm the results in cases referred. Apart from laboratory

error, two sources of falsely high plasma calcium levels require consideration. There is often a small but significant rise in the plasma calcium level soon after the ingestion of calcium, and the plasma calcium levels of a group of individuals have a narrower range after a standard overnight fast, so that blood should always be collected under these standard conditions (Dent, personal communication). Secondly, the influence of hæmostasis during collection must be taken into account. A few subjects quickly produce hæmoconcentration after venous occlusion, presumably by "ultrafiltering" their plasma through the capillary wall into the extravascular spaces (Dent, personal communication). Therefore blood should be taken with a minimum of venous occlusion, and the plasma proteins (or the plasma specific gravity) should be checked, for in these cases it is the protein-bound fraction which is increased.

REFERENCES

ALBRIGHT, F., AUB, J. C., and BAUER, W. (1934), "Hyperparathyroidism: A Common and Polymorphic Condition as Illustrated by Seventeen Proved Cases from One Clinic", *J. Amer. med. Ass.*, **102**, 1276.

ALBRIGHT, F., and ELLSWORTH, R. (1929), "Studies on the Physiology of the Parathyroid Glands. I: Calcium and Phosphorus Studies on a Case of Idiopathic Hypoparathyroidism", *J. clin. Invest.*, **7**, 183.

ALBRIGHT, F., and REIFENSTEIN, E. C. (1948), "The Parathyroid Glands and Metabolic Bone Disease", Williams & Wilkins, Baltimore.

BARNICOT, N. A. (1948), "Local Action of Parathyroid and Other Tissues on Bone in Intracerebral Grafts", *J. Anat.*, **82**, 233.

CHAMBERS, E. L., JR, GORDON, G. S., GOLDMAN, L., and REIFENSTEIN, E. C., JR (1956), "Tests for Hyperparathyroidism: Tubular Reabsorption of Phosphate, Phosphate Deprivation and Calcium Infusion", *J. clin. Endocr.*, **16**, 1507.

COLLIP, J. B., CLARK, E. P., and SCOTT, J. W. (1925), "The Effect of a Parathyroid Hormone on Normal Animals", *J. biol. Chem.*, **63**, 439.

DENT, C. E. (1956), "Cortisone Test for Hyperparathyroidism", *Brit. med. J.*, **1**, 230.

DENT, C. E. (1960), personal communication.

DENT, C. E., BISHOP, P. M. F., and DE MOWBRAY, R. R. (1953), "Discussion on Physiology and Clinical Disorders of the Parathyroid Glands", *Proc. roy. Soc. Med.*, **46**, 291.

DENT, C. E., and GARRETTS, M. (1960), "Skin Changes in Hypocalcæmia", *Lancet*, **1**, 142.

DENT, C. E., PYRAH, L. N., and STEWART, H. H. (1956), "Discussion on Surgical Aspects of Disordered Calcium Metabolism", *Proc. roy. Soc. Med.*, **49**, 715.

DICKENS, F. (1941), "The Citric Acid Content of Animal Tissues, with Reference to its Occurrence in Bone and Tumour", *Biochem. J.*, **35**, 1011.

FANCONI, A., and ROSE, G. A. (1958), "The Ionised, Complexed and Protein-bound Fractions of Calcium in Plasma; An Investigation of Patients with Various Diseases which Affect Calcium Metabolism, with an Additional Study of the Role of Calcium Ions in the Prevention of Tetany", *Quart. J. Med.*, **27**, 463.

GRANT, D. K. (1953), "Papillœdema and Fits in Hypoparathyroidism, with a Report of Three Cases", *Quart. J. Med.*, **22**, 243.

HODGKINSON, A., and PYRAH, L. N. (1958), "The Urinary Excretion of Calcium and Inorganic Phosphate in 344 Patients with Calcium Stone of Renal Origin", *Brit. J. Surg.*, **46**, 10.

KNAPP, E. L. (1947), "Factors Influencing the Urinary Excretion of Calcium. I. In Normal Persons", *J. clin. Invest.*, **26**, 182.

LITVAK, J., MOLDAWER, M. P., FORBES, A. P., and HENNEMAN, P. H. (1958), "Hypocalcæmic Hypercalciuria during Vitamin D and Dihydrotachysterol Therapy of Hypoparathyroidism", *J. clin. Endocr.*, **18**, 246.

LLOYD, H. M., and ROSE, G. A. (1958), "Ionised Protein-bound and Complexed Calcium in the Plasma in Primary Hyperparathyroidism", *Lancet*, **2**, 1258.

NEUMAN, W. F., FIRSCHEIN, H., CHEN, P. S., MULRYAN, B. J., and DI STEFANO, V. (1956), "On the Mechanism of Action of Parathormone", *J. Amer. chem. Soc.*, **78**, 3863.

NEUMAN, W. F., and NEUMAN, M. W. (1958), "The Chemical Dynamics of Bone Mineral", Univ. Chicago Press, Chicago.

ROSE, G. A. (1957), "Determination of the Ionised and Ultrafilterable Calcium of Normal Human Plasma", *Clin. chim. Acta*, **2**, 227.

SUGAR, O. (1953), "Central Neurological Complications of Hypoparathyroidism", *A.M.A. Arch. Neurol.*, **70**, 86.

THOMAS, W. C., JR, CONNOR, T. B., and MORGAN, H. G. (1958), "Some Observations on Patients with Hypercalcæmia Exemplifying Problems in Differential Diagnosis, Especially in Hyperparathyroidism", *J. Lab. clin. Med.*, **52**, 11.

THOMAS, W. C., JR, CONNOR, T. B., and MORGAN, H. G. (1959), "Diagnostic Considerations in Hypercalcæmia with a Discussion of the Various Means by which such a State may Develop", *New Engl. J. Med.*, **260**, 591.

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